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Proceedings of the Conference on the Diaporthe/Phomopsis Disease Complex of Soybean

March 26-27, 1984
Fort Walton Beach, Florida

PREFACE

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The recent widespread outbreak of soybean stem canker in many Southeastern States has caused a resurgence of interest in the Diaporthe/Phomopsis disease complex. This conference was organized to act as a forum for scientists working on various aspects of this disease complex. The conference attracted an audience of approximately 110 who heard 23 papers presented by 21 scientists from a number of States, Brazil, Canada, and Italy. In addition, the conference included opening remarks by Dr. Howard E. Waterworth, ARS-USDA, Beltsville, MD, a report on the Birmingham, AL, stem canker meeting of November 3-4, 1983, by Dr. Daniel V. Phillips of the Georgia Agricultural Experiment Station, Experiment, and a 10-member panel discussion of stem canker, chaired by Dr. William F. Moore, Mississippi State University. Many people contributed to the success of this conference, including Dr. James D. Arnett, Georgia Coastal Plain Experiment Station, Tifton, Dr. Paul A. Backman, Auburn University, Dr. Richard E. Stuckey, University of Kentucky, and Dr. Howard E. Waterworth. Thanks are also due to the Southern Soybean Disease Workers for allowing this conference to be held in conjunction with their annual meeting.

Martin M. Kulik
Editor and
Conference Organizer

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SUMMARY

Fungi of the Diaporthe/Phomopsis disease complex of soybean cause seed decay or mold and stem canker, which can result in serious reductions in seed quality and crop yields. At the conference on this disease complex, reports of research on various aspects of these two problems were presented. In the first session, "The Causal Fungi," Morgan-Jones of Auburn University discussed the morphology of members of this fungus complex. Strains from different geographical areas were found to differ somewhat in their in vitro cultural characteristics. He also reported that variation exists in stromatal size and in the shape of perithecia and ascospores. Kulik of the U.S. Department of Agriculture, Beltsville, MD, reported that Phomopsis phaseoli penetrates soybean cotyledons, leaves, and hypocotyls via the stomates or by direct penetration of the cuticle by means of an appressorium. He also discussed the isolation from stem cankers of strains of Diaporthe phaseolorum f. sp. caulivora with atypical ascospores that were fusoid, often curved, seemingly three septate but actually only one septate, and that resembled in shape the conidia of Bipolaris. Mannerucci and Gambogi of the University of Pisa, Italy, found that certain morphological characteristics among biotypes of Phomopsis may not be related to certain physiological activities. Culture filtrates of certain strains of this fungus inhibited the germination of soybean seeds and caused necrosis of cuttings of soybean, chickpea, broadbean, pea, green bean, and lupine. Hobbs and Schmitthenner of the Ohio Agricultural Research and Development Center, Wooster, OH, used scanning electron and light microscopy to study anamorphs of Phomopsis isolated from soybean plants. These isolates could be assigned to two species, P. sojae and an unnamed, new species of Phomopsis, based on their growth on acidified potato dextrose agar.

In the session on "Epidemiology," Sinclair of the University of Illinois discussed the epidemiology of fungi of the Diaporthe/Phomopsis disease complex, based on studies conducted since 1970 in Illinois, Brazil, and Puerto Rico. Moisture, more than temperature, cultural practices, or geographic area, had the most influence on the occurrence of this disease. Seed infection was found to be greater in the lower pods than in the higher pods, with susceptibility directly related to pod age. Coinfection of seeds by other fungi reduced infection by Phomopsis. Gleason and Ferriss of the University of Kentucky studied the effects of soil moisture level on seedling emergence and establishment from Phomopsis-infected seeds. These effects were most pronounced in dry soil. The influence of environmental factors on the movement of Phomopsis from pods into seeds was studied by Balducchi and McGee of Iowa State University. Their results showed that the amount of seed infection by this fungus is directly related to the amount of podborne inoculum and, also, to high temperature and relative humidity during the infection period. McGee also presented details of his method for using the amount of podborne inoculum to predict Phomopsis seed decay and the need for applying fungicidal sprays. Thomison, Jeffers, and Schmitthenner of the Ohio Agricultural Research and Development Center, Wooster, studied the influence of pod nutrient content on Phomopsis infection of pods and seeds. Thomison reported their finding that increased infection and reduced seed germination, which resulted from smaller numbers of pods per plant, were associated with a slower rate of plant maturation and drying of seeds, higher levels of total nonstructural carbohydrates, nitrogen, and phosphorus in pod walls, and larger and heavier pod walls and seeds. Rupe and Ferriss of the University of Kentucky studied the effects of moisture on infection of soybean seeds by Phomopsis. Their results suggested

that the rate of seed infection does not change as the moisture level changes but that moisture levels from 20 to 40 percent warrant more investigation. They reported that this fungus can infect seed under very dry conditions (that is, 20 percent moisture); the growth rate of Phomopsis on potato dextrose agar plus sucrose or KCl increased rapidly as the osmotic potential was reduced from -3 to -10 bars, but was optimal at from -10 to -30 or -40 bars, and reached an end point at about -180 to -190 bars; the optimum rate of seed infection coincided with water potentials associated with the optimal growth rate of the fungus; and the close correlation between the growth of Phomopsis in vitro and seed infection may indicate that the water potential of the pod is the chief factor involved in seed infection at moderate temperatures once physiological maturity is reached. The infection of pods and seeds by Phomopsis during soybean seed development and maturation was studied by Tomes, Hicks, and TeKrony of the University of Kentucky. Pod infection by this fungus began early in seed development and was greater than 90 percent at physiological maturity of the pods. Senescence was directly associated with pod colonization by Phomopsis. Little or no seed infection occurred when pods were green or yellow; but, subsequently, in periods of high temperature and moisture, there was a rapid increase in seed infection. It appeared that invasion of the seedcoat by Phomopsis occurred directly from the pod.

In the session on "Effects on Seed Quality," Hill, West, and Hinson of the University of Florida presented results of a study on infection of soybean seeds differing in permeability. They believe that pores in the seedcoat may provide the means of entry for fungi into seeds that are free of any visible defects in the seedcoat. Impermeable seeds thus may have an effective mechanical barrier to fungal penetration compared to seeds with normal seedcoats. This resistance to invasion, plus retention of viability, persisted in impermeable seeds subjected to delayed harvest. This study supports the concept that improved seedcoats may offer a practical means of maintaining soybean seed quality. Anderson and Buzzell of the Agriculture Canada Research Station at Harrow, Ontario, studied the heritability of tolerance to Diaporthe/Phomopsis seed mold of soybeans. They concluded that there are differences in susceptibility to this disease among soybean lines; heritabilities in the broad sense were sufficiently high to allow selection, but the magnitude of the variety x year interactions indicated a need for data over years and/or locations also; more research is needed to determine the suitability of using late harvest and irrigation in selecting seed-mold-tolerant cultivars that differ in their maturity requirements; the effect of maturity date on the incidence of seed mold needs more investigation, and, in this regard, the evaluation of isolines that differ in their maturity requirements may be of use. Phillips of the Georgia Agricultural Experiment Station, Experiment, and Guerke of the Georgia Department of Agriculture Seed Laboratory, Atlanta, studied the effect of Diaporthe/Phomopsis infection on the reproducibility of soybean germination tests. Using data obtained from 30 laboratories that took part in an Association of Official Seed Analysts' referee test, they found that variation in the results of these tests, both within and between laboratories, ran much higher for seed lots which contained high levels of infection by these fungi. Schoen of the U.S. Department of Agriculture, Beltsville, MD, presented a report on disease symptoms that can appear in germination tests of soybeans. The symptoms produced by Phomopsis and several other seedborne fungi were described, and guidelines were presented for evaluating the germination of infected seed lots. Henning and França Neto of the National

Center for Soybean Research, Londrina, Parana, Brazil, found that infection of soybean seeds by Phomopsis did not affect seedling emergence under conditions of adequate soil moisture and temperature. This fungus was mainly confined to the seedcoats, and the emerging seedlings usually escaped the pathogen by leaving the seedcoats in the soil. Also, Phomopsis died out in stored seeds relatively rapidly, resulting in an apparent increase in seed viability.

In the session on "Control Measures," Stuckey, Tomes, TeKrony, and Egli of the University of Kentucky evaluated the Kentucky Point System for scheduling applications of benomyl to soybeans being grown for seed. Based on the results of tests of commercial fields and University test plots, the Kentucky Point System was found to be more accurate in predicting those fields that would benefit from benomyl application than point systems developed in other States and by industry. In this method, the grower assigns point values to four parameters: field cropping history, maturity date of cultivar, planting date, and amount of rainfall. The addition of a fifth parameter, the amount of pod infection at growth stage R-6, may be of value for those fields whose point totals do not clearly fall within the "apply" or "do not apply" categories. Pacumbaba, Sapra, and Prom of Alabama A & M University, conducted a study of the effect of two commercial fungicides on the incidence of stem canker on susceptible soybean cultivars. One application of Manzate 200 or Dyrene before inoculation with the stem canker pathogen resulted in significantly better control of the disease than one application after inoculation. Significantly higher yields also were obtained by spraying prior to rather than after inoculation. Springer of the Rutgers Research and Development Center, Bridgeton, NJ, and Halisky of Rutgers University studied the effect of Diaporthe and other seedborne fungi on the germination of soybean seeds and the survival of seedlings. In 1977, seed lots from control plots showed 43 percent moldy beans due to Diaporthe, and the germination of seeds from these lots was zero. In 1983, the application of foliar fungicides did not improve germination, seedling vigor, or control of damping-off. Backman of Auburn University discussed his research on the effects of timing and rates of application of fungicides for the control of stem canker. Frequent spraying beginning as early as V-2 was necessary to insure adequate disease control in cultivars of intermediate susceptibility to this disease. As many as five applications were not effective in controlling stem canker in highly susceptible cultivars. Crawford of Auburn University reported on a study he conducted of seed treatments and tillage practices as they affect the spread and control of stem canker. Seed treatment fungicides were seen to be effective in reducing the amount of seedborne infection. Comparisons between conventional and no-tillage cultivation systems indicated that soybeans grown under the former had more stem canker than plants grown under the latter system. The soybeans in the conventional tillage plot developed a denser canopy which may have contributed to the incidence of disease. Weaver of Auburn University studied the effects of cultivar and planting date on the incidence of stem canker in the field. Of the forty-seven cultivars evaluated during 1982 and 1983, only Tracy M and Braxton had superior resistance to this disease. 'Braxton' outyielded all the other cultivars, but the yield of 'Tracy M' did not differ significantly from the yields of several cultivars that had higher disease ratings. The incidence of stem canker was highest for the cultivars Wilstar 790 and Hutton. It is suggested that delayed planting may reduce losses due to stem canker, particularly in the more susceptible cultivars. However,

other production factors may cancel out the increased yields seen in delayed planting. Bolkan of the University of Brazil was not able to attend the conference. His paper dealt with work on the prevalence and control of seedborne Phomopsis in his country. The incidence of this fungus in soybean seeds was found to be related to year, growing region, and cultivar. The fungus was detected with about the same frequency in the seedcoat and in the embryo, and higher recoveries were obtained on blotters than on potato dextrose agar. There were significant differences in the amount of Phomopsis recovered from various plant parts. The percentage of seed germination in vitro and field emergence were each negatively correlated with the percentage of seedborne Phomopsis. Seed treatment with fungicides resulted in a significant increase in germination and seedling emergence. Fungicide sprays likewise significantly reduced the amount of seedborne Phomopsis.

OPENING REMARKS

Howard E. Waterworth[1]

The stem canker disease of soybeans certainly received a lot of attention during 1983, especially in the South. From my vantage point on the National Program Staff of ARS, it vied for attention along with citrus canker and the karnal bunt disease of wheat.

The dramatic increase in stem canker has caused considerable and justifiable concern by growers and commodity groups and has had the attention of the press in the South. It has been the subject of at least two meetings here in the South--one called by James Carpenter, Director of the Mississippi Cooperative Extension Service, the other a regional meeting in Birmingham last November. My interest and concern stem not only because ARS has a role to play in solving the problem, but frankly also because of Congressional inquiries that ARS has been responding to, among other reasons.

Estimates of losses range all over the board. One report estimated losses at $36.5 million during 1983 in a seven-State area. The Southern Soybean Disease Workers (SSDW) placed losses during 1982 at $105 million in a 14-State area in the Southeast. If, on a national basis, losses are only 1 percent, we are still talking about a major problem when one considers that soybeans have a production value of over $13 billion. Furthermore, it is not clear whether the loss estimates are only the value of lost production or whether they include the substantial additional costs of attempting to control the disease. A comprehensive 1979 study of disease losses in North Carolina, for example, showed that costs of controlling stem canker were 8.4 times as much as the value of lost crop. Clearly, then, we are addressing a problem of major economic importance.

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What are the purposes or objectives of this special meeting? I believe they include a general assessment of the problems to be dealt with; obviously, an exchange of information on where we are now in solving these problems; and a better appreciation by all of us on what some of the current priority approaches might be in dealing with stem canker.

One of the contributions that ARS will make is in the area of germplasm. Many have said that ARS should have a major role in collecting, maintaining, evaluating, and enhancing germplasm for all crops. We accept this responsibility and have been successful in recent years in getting increased appropriations to expand these programs. In soybeans, for example, permanent increases in base funding were made in our programs at Stoneville and Urbana for maintenance and evaluation work.

With regard to collecting germplasm, soybeans are among the highest priority crops for which a foreign collection is needed. And, if the People's Republic of China would allow a team to collect germplasm freely, rather than on their restrictive terms, ARS would take quick action to accomplish this.

In the realm of breeding, some of you may have heard the rumor that ARS is ceasing plant breeding work. Let me assure you that this is not correct. ARS will always be making important contributions to improving crops on those aspects that the private sector cannot afford to pursue--such as developing new ways to manipulate genes, enhancing germplasm, and even conventional breeding on some crops. In fact, resistance to soybean canker has been an important part of the ARS breeding program in recent years.

With regard to pathology and nematology, we see our role leaning more toward long-term, higher risk basic research:

developing new approaches to disease control such as induced resistance, bioregulation, elucidating the biochemical mechanisms that are responsible for resistance or susceptibility, and so forth. I also want you to know that pathology and nematology are among the programs scheduled for increased support in our 6-year plan.

I look forward to meeting you and hearing your research reports. Finally, I want to thank Martin M. Kulik for his leadership in organizing this meeting and the other leaders of the SSDW for allowing us to meet with them.

SESSION A. THE CAUSAL FUNGI

Kenneth W. Roy, Chairman[1]

The *Diaporthe*/*Phomopsis* Complex of Soybeans: Morphology

Gareth Morgan-Jones[2]

Within the *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. [= *Sphaeria phaseolorum* Sacc.] species concept, four entities have been recognized and given varietal taxonomic rank. These have largely been based upon host-parasite association. Morphologically there are few, if any, differences among them. The lectotype variety, var. *phaseolorum* occurs on lima beans [*Phaseolus lunatus* L.] whilst var. *batatatis* (Harter & Field) Wehm. [= *Diaporthe batatatis* Harter & Field] is the cause of dry rot of sweet potatoes [*Ipomoea batatas* (L.) Lam.]. Varieties *caulivora* Athow & Caldwell and *sojae* (Lehm.) Wehm. [= *Diaporthe sojae* Lehman] occur on soybean [*Glycine max* (L.) Merr.], causing stem canker and pod and stem blight, respectively.

Wehmeyer (1933) recognized the similarity of the three teleomorphs *D. phaseolorum*, *D. batatatis* and *D. sojae* when he transferred the last two specific epithets to varietal rank within the former. He stated that all three were morphologically very similar and probably fully conspecific. Kulik (1984) concurred with this assessment and went even further by advocating the adoption of but one binomial for the three taxa and dispensing with the varietal names. The valid name, based on nomenclatural date priority, is *D. phaseolorum*. *D. batatatis* and *D. sojae*, and the varietal entities based on the recombination of these specific epithets, should therefore be treated as facultative synonyms of *D. phaseolorum*. *D. phaseolorum* is considered to be plurivorous in habit, but within it there are no stable morphological discontinuities sufficient to warrant separate, formal, taxonomic status for biotypes occurring on different hosts and inducing differing disease syndromes.

Diaporthe phaseolorum var. *caulivora* was established by Athow and Caldwell (1954) and differentiated from var. *sojae* on the basis of the absence of an associated anamorph, occurrence of perithecia in caespitose clusters rather than singly, possession of shorter and more tapering perithecial beaks and smaller asci and ascospores. Welch and Gilman (1948) had previously found isolates from soybean cankers (which they determined to be a strain of var. *batatatis*) to differ from var. *sojae* in pathogenicity and type of resultant disease induction, homothallism, possession of caespitose perithecia, and absence of pycnidia. Although the validity of some of these distinctions has, by and large, been accepted, some question exists as to their taxonomic significance. Threinen et al. (1959) obtained radiation-induced mutants of both vars. *caulivora* and *sojae* that differed from parental types in morphology, pathogenicity, and genetic behavior. Whitehead (1966), who documented stem canker induction on soybean and birdsfoot trefoil (*Lotus corniculatus* L.) infected by var. *sojae*, reached a similar conclusion to the one of Threinen et al. that the two varieties were insufficiently distinct to justify separate taxonomic rank. Schmitthenner and Kmetz (1980) added credence to this view when they reported that occasional isolates of var. *sojae* were able to produce canker-like symptoms. Added to this, Hildebrand (1954) noted that occurrence of perithecia of var. *caulivora* in caespitose clusters was not always constant, and both he and

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Frosheiser (1957) found pycnidia in isolates from soybean stem canker.

There appear to be insufficient grounds for maintenance of var. caulivora as a separate taxonomic entity (Kulik 1984), especially given that the other three varieties are considered superfluous and therefore redundant. The strains associated with stem canker can, however, as Kmetz et al. (1979) have pointed out, be consistently distinguished from others of D. phaseolorum. To accommodate this fact, Kulik (1984) has advocated that strains causing soybean stem canker be referred to, at least provisionally, as forma specialis caulivora to indicate association with a distinct disease condition. This treatment recognizes that the distinction mainly involves physiological reaction, although this may be accompanied by slight morphological differences, especially in cultural characteristics in vitro.

Typically, isolates of D. phaseolorum originating from soybeans and associated with pod and stem blight symptoms (that is, those referred to var. sojae in the past) have colonies which tend to become funiculose, bearing radiating strands of hyphae which give a dark striate appearance in reverse and which become pigmented with age. Such strains, given the right conditions, almost invariably readily produce an anamorph state. Stromatic tissue is formed from which pycnidia and, subsequently, perithecia are produced. The stroma consists of a pseudoparenchymatous ectostroma and a plectenchymatous entostroma. Short pycnidial beaks emerge through the compact ectostroma as do the longer perithecial necks. The pycnidia produce both alpha and beta conidia (fig. 1B), the type produced depending on nutritional factors. Beta conidia are considered to be relictual spermatia, and nutrient depletion causes a shift from true conidia (alpha conidia) to the spermatia.

Isolates associated with stem canker, referable to f. sp. caulivora, rarely produce an anamorph state in vitro. When present, pycnidia sometimes bear both alpha and beta conidia, but many strains produce only beta conidia. Perithecia are readily produced in some abundance. Whereas those isolates traditionally named var. sojae show some stability in their cultural characteristics, those of f. sp. caulivora vary considerably depending, it seems, upon their geographical origin. Within this forma specialis, Keeling (1984), following comparison of isolates from different geographical locations and a study of their pathogenicity to various soybean cultivars, determined the presence of six physiologic races. He suggested that they can be differentiated by the cultivars Kingwa, Tracy-M, Arksoy, Centennial, S-100, and J77-339. Southern isolates were designated races 1, 2, or 3; and northern isolates, races 4, 5, or 6. It was observed, using the toothpick inoculation method, that northern cultivars were susceptible to southern isolates. Isolates originating in Indiana, Iowa, and Ohio are more or less identical in their growth characteristics. Fourteen-day-old colonies on potato dextrose agar (PDA) are white and thinly lanose, bearing highly typical, small, evenly scattered, dense tufts of hyphae. The numerous white tufts give the colonies a somewhat mottled appearance. Small perithecial stromata are evident as black dots in the colony reverse. Each stroma forms a group of perithecia whose necks appear in caespitose clusters above the hyphal surface. Perithecial necks are tenuous, somewhat flexuous and attenuated to sharply acute apex (fig. 2B). Isolates originating in Alabama, Georgia, and Mississippi are quite different in colony appearance and very variable. Fourteen-day-old colonies on PDA are whitish to cream to peach to pinkish in coloration and usually distinctly felted, becoming progressively more so with age. Some colonies have irregular patches of

pinkish coloration intermixed with cream to buff areas. Stromata in most of the southern isolates are appreciably larger than those in the northern strains studied and appear as black, randomly scattered areas in colony reverse. Perithecial necks produced from these stromata are generally rather stouter (fig. 2C). Some of the strains isolated in the South grow much more rapidly than northern isolates. Whether or not this is a consistent differentiating feature remains to be determined, however.

Collections of f. sp. caulivora made on soybean stems in Alabama frequently show perithecia to be gregarious within clearly defined areas bordered by thin bands of black stromatic tissue (fig. 2A). Within these areas the periderm appears whitish. Occasional perithecia occur outside such areas. The perithecia are, in most instances, solitary and more or less evenly scattered (fig. 2B). This pattern of perithecial production differs from the caespitose arrangement noted by Athow and Caldwell (1954). The perithecial necks are long (up to 1,500 µm in length), robust, and more or less straight. An additional teleomorph variant characteristic encountered in Alabama collections concerns ascospore shape. In many specimens, ascospores are broadly fusiform, obtuse at each end, straight or frequently somewhat allantoid, and unconstricted in the vicinity of the median septum (fig. 3). In typical f. sp. caulivora ascospores, as described by Athow and Caldwell (1954), a slight constriction occurs at the septum; the ends are more acute; and there is little, if any, tendency to curve.

The anamorph states of subspecific divisions of D. phaseolorum, to which the names Phomopsis batatae Harter and Field, P. phaseoli (Desm.) Grove (Phoma phaseoli Desm.), and P. sojae Lehman have been applied, were considered by Kulik (1984) to represent one taxon, the valid name for which is P. phaseoli. As in the case of the teleomorph, there are insufficient distinguishing characteristics to justify their classification in separate taxa.

There exists on soybeans a Phomopsis anamorph very different in morphological characteristics in vitro from the anamorph of D. phaseolorum (that is, P. phaseoli). The organism seems to be predominantly associated with seed decay. It was reported by Kmetz et al. (1974) to be more highly pathogenic to seed then P. phaseoli. Its most noticeable feature is production of large, somewhat pulvinate stromata which bear distinctly rostrate pycnidia (fig. 1A). In older cultures inflated, chlamydospore-like cells occur (fig. 1B) and alpha conidia are somewhat variable in shape (fig. 1C). Beta conidia are rarely formed and no teleomorph connection is known. This entity is considered distinct enough to warrant accommodation in a separate species for which the name Phomopsis longicolla Hobbs apud Hobbs et al. (1984) is being established.

Diaporthe phaseolorum is a highly versatile entity in terms of host range and disease inducing capacity. Considerable genetic diversity, reflected in both morphological variation and physiological specialization, is exhibited among its biotypes. In different areas of the country, given varying selection pressures (climatic factors, host peculiarities--particularly resistance/susceptibility factors among cultivars--and so forth), different biotypes predominate.

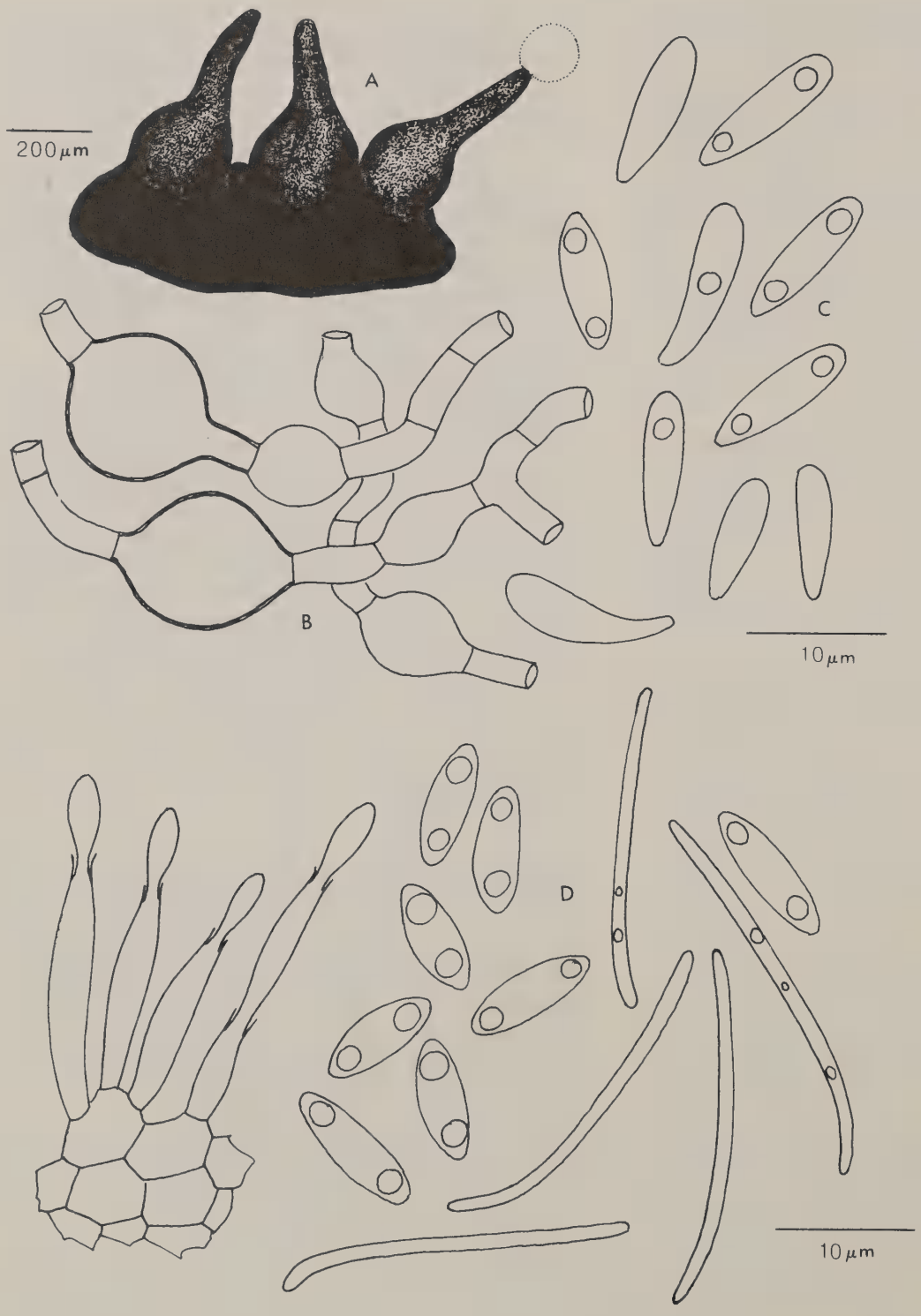

Figure 1.--Phomopsis longicolla pycnidia, chlamydospores, and conidia (A-C) and Phomopsis phaseoli conidiophores and conidia (D).

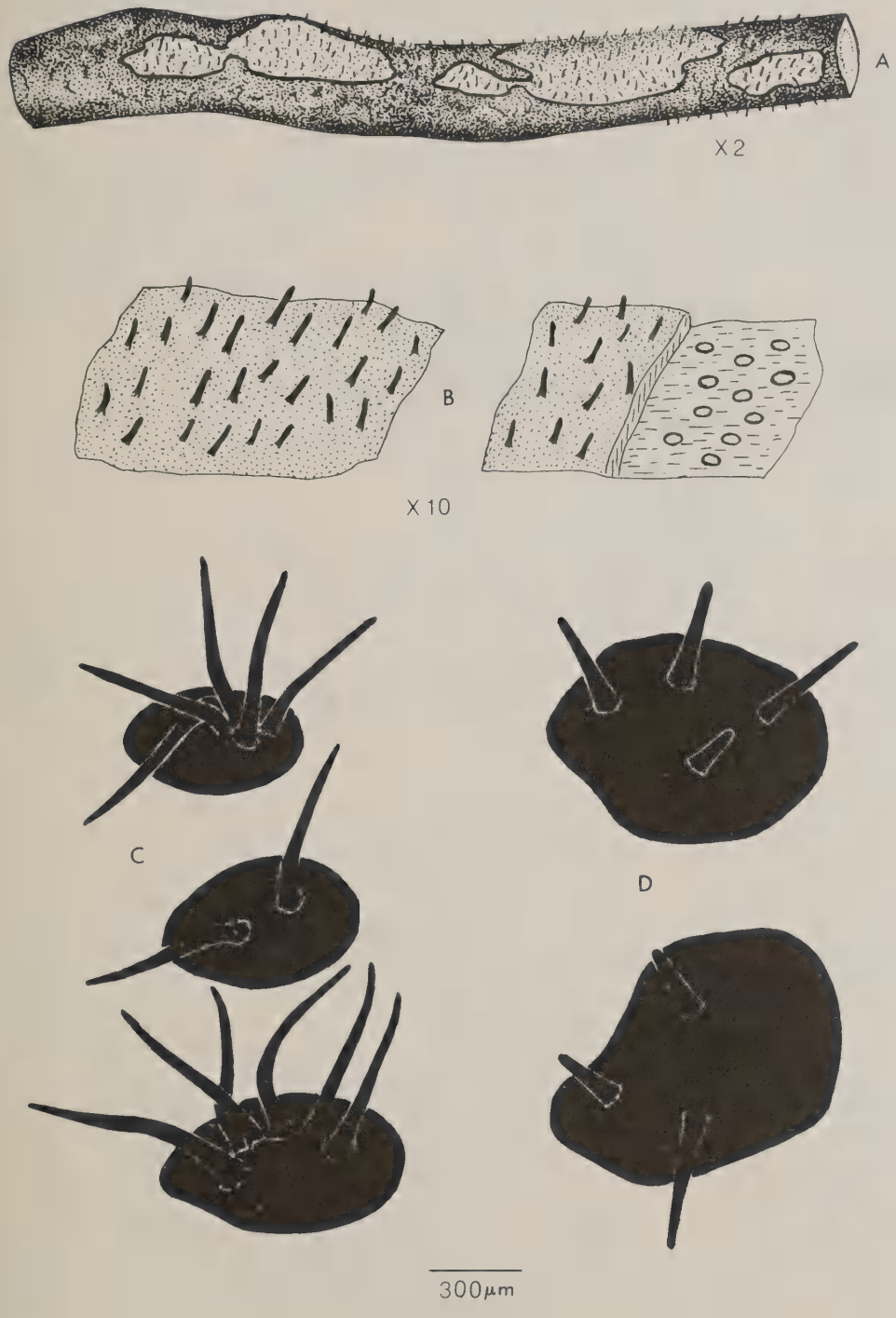

Figure 2.--Diaporthe phaseolorum f. sp. caulivora perithecia on soybean stem (A,B--Alabama) and perithecial clusters in vitro (C--Iowa isolate, D--Mississippi isolate).

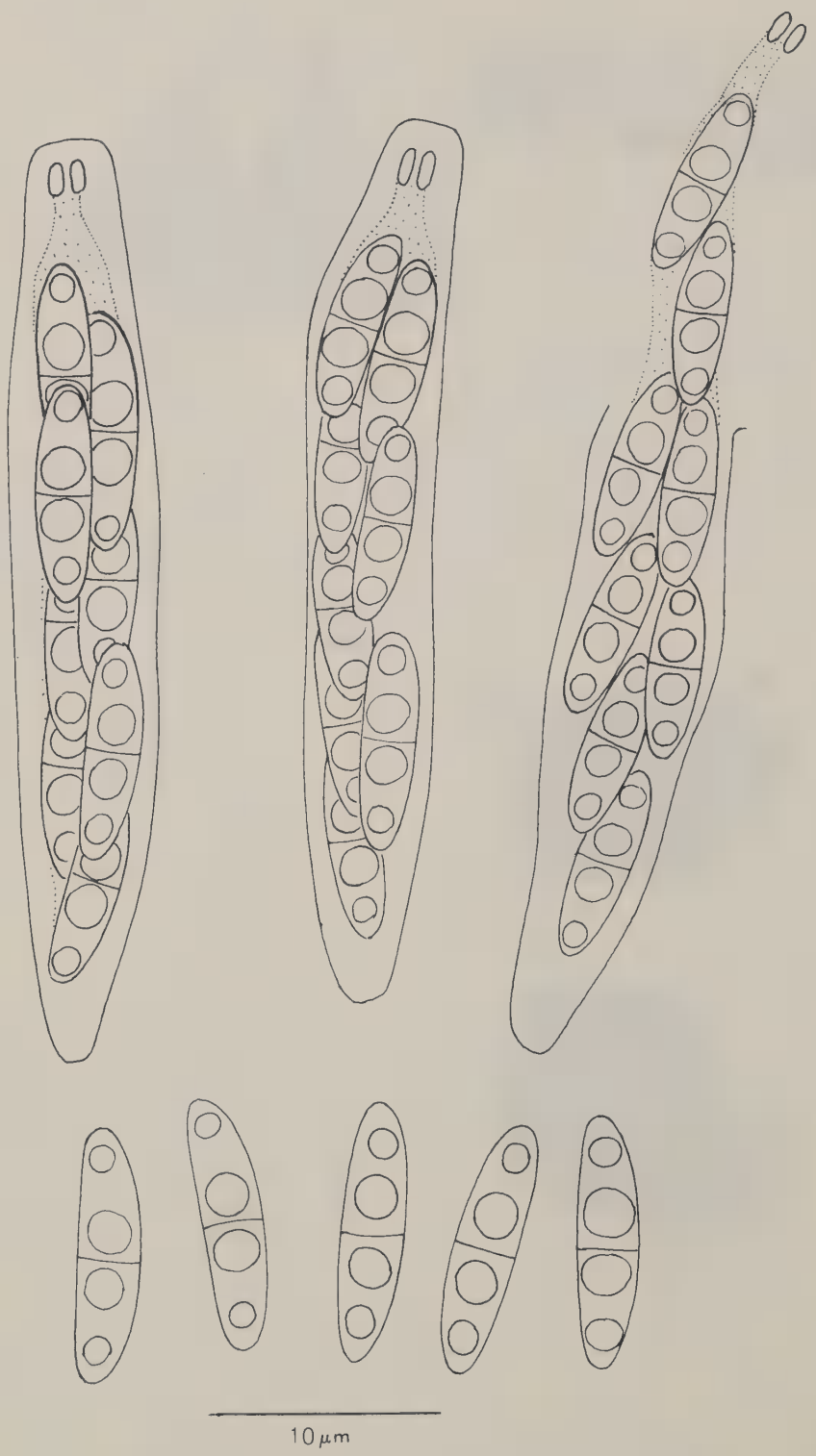

Figure 3.--D. phaseolorum f. sp. caulivora asci and ascospores of Alabama collection.

References

Athow, K. L. and R. M. Caldwell.
1954 A comparative study of Diaporthe stem canker and pod and stem blight of soybeans. Phytopathology 44:319-325.

Frosheiser, F. I.
1957 Studies on the etiology and epidemiology of Diaporthe phaseolorum var. caulivora, the cause of stem canker of soybeans. Phytopathology 47:87-94.

Hildebrand, A. A.
1954 Observations on the occurrence of the stem canker and pod and stem blight fungi on mature stems of soybean. Pl. Dis. Rep. 38:640-646.

Hobbs, T. W., A. F. Schmitthenner, and
1984 G. A. Kuter. A new Phomopsis species from soybean. Mycologia 76 (in press).

Keeling, B. L.
1984 Evidence for physiologic specialization in Diaporthe phaseolorum var. caulivora. J. Miss. Acad. Sci. Suppl. 29:5.

Kmetz, K. T., C. W. Ellett, and
1974 A. F. Schmitthenner. Isolation of seedborne Diaporthe phaseolorum and Phomopsis from immature soybean plants. Plant Dis. Rep. 58:798-801.

Kmetz, K. T., C. W. Ellett, and
1979 A. F. Schmitthenner. Soybean seed decay: sources of inoculum and nature of infection. Phytopathology 69:798-801.

Kulik, M. M.
1984 Symptomless infection, persistence, and production of pycnidia in host and non-host plants by Phomopsis batatae, Phomopsis phaseoli, and Phomopsis sojae, and the taxonomic implications. Mycologia 76:274-291.

Schmitthenner, A. F. and K. T. Kmetz.
1980 Role of Phomopsis sp. in the soybean seed rot problem. In F. T. Corbin, editor, World Soybean Res. Conf. II, p. 355-366. Westview Press, Boulder, CO.

Threinen, J. T., T. Kommedahl, and
1959 R. J. King. Hybridization between radiation-induced mutants of two varieties of Diaporthe phaseolorum. Phytopathology 49:797-801.

Wehmeyer, L. W.
1933 The genus Diaporthe Nitschke and its segregates. p. 349. Univ. Michigan Press, Ann Arbor.

Welch, A. W. and J. C. Gilman.
1948 Hetero- and homothallic types of Diaporthe of soybeans. Phytopathology 38:628-637.

Whitehead, M. D.
1966 Stem canker and blight of birdsfoot-trefoil and soybean incited by Diaporthe phaseolorum var. sojae. Phytopathology 56:396-400.

Preliminary Report on the Mode of Penetration of Soybean Vegetative Tissues by *Phomopsis phaseoli* (syn. *P. sojae*)

Martin M. Kulik[1]

Using scanning electron microscopy (SEM) and freeze-fracturing, it appeared that *Phomopsis phaseoli* (Desm.) Grove (syn. *P. sojae* Lehm.) penetrates soybean [*Glycine max* (L.) Merr.] cotyledons, leaves, and hypocotyls in two ways: via the stomates or by direct penetration of the cuticle by means of an appressorium.

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Preliminary Report on the Occurrence of Isolates of *Diaporthe phaseolorum* f. sp. *caulivora* (syn. var. *caulivora*) with Atypical Ascospores

Martin M. Kulik

The stem canker pathogen, *Diaporthe phaseolorum* (Cke. & Ellis) Sacc. f. sp. *caulivora* (Athow & Caldwell) Kulik (= var. *caulivora* Athow & Caldwell), was described by Athow and Caldwell as having ascospores that were elongate-ellipsoidal, two-celled, with two guttules per cell, and slightly constricted at the septum. We now report the isolation of strains of this fungus from cankered soybean [*Glycine max* (L.) Merr.] stems from Alabama and Georgia, and from noncankered stems from Maryland, with ascospores that were fusoid, often curved, seemingly three septate (but actually only one septate), resembling in shape the conidia of *Bipolaris* Shoemaker. Isolates of *D. phaseolorum* f. sp. *caulivora* with fusoid ascospores predominated in the infected material from the Deep South.

In Vitro Morphological and Physiological Aspects of *Phomopsis* from Soybean Seed

G. Favilli Mannerucci and Piero Gambogi[1]

Most morphological aspects of the *Diaporthe*/*Phomopsis* complex are known either on the plant or in culture. Both the teleomorphs and the anamorphs are seedborne and affect the quality of seeds, reducing their germination and vigor. If the teleomorph is absent, the anamorph should be referred to by the genus name only. Many described species of *Phomopsis* exhibit the features of the whole genus, and possibly the one(s) found on soybean belong to these.

Four isolates of *Phomopsis*, A, B, C, and F, differing in colony morphology, conidiomata shape and A/B spore ratio, were obtained from one seed sample of soybean from an area of limited cultivation in central Italy. The main characteristics of these strains can be briefly described as follows: A--mycelium dense, thin-layered and superficial; stromatal bodies, black, crust-like; conidiomata globose to flattened, borne in depressions of the stromatal body; conidia almost totally B conidia; B--mycelium scanty; conidiomata scattered, globose to subglobose; conidia almost totally A conidia; C--mycelium dense, thin-layered; conidiomata uni-plurirostrate, scattered or grouped; conidia mostly A conidia; F--mycelium luxuriant; conidiomata rare, globose, and sunken in the mycelium; conidia almost totally A conidia. The aim of the present study is to provide information on some of the physiological properties shown by these isolates.

Isolations were made from soybean seeds after surface sterilization (sodium hypochlorite) and incubation on potato dextrose agar. A first set of monosporic isolations was followed by serial massive spore transfers (spore drops, tendrils) to Difco PDA, being careful that selected characters were maintained each time. All the strains were used to inoculate soybean plants (cv. Amsoy) with the toothpick method, 15 days after sowing, using healthy seeds in steamed soil in pots in the greenhouse, 22 to 25°C. In the greenhouse, 10 days after inoculation, a limited brown discoloration of tissues developed around the point of infection. Later on, there was a tendency of the areas to become necrotic. This was more evident for the F strain.

In order to establish whether fungus metabolites could be involved in the disease syndrome, in addition to direct invasion and deterioration of tissues, cell-free culture filtrates were used in germination tests and a cutting bioassay.

Stationary cultures on Czapek's broth (pH 6.5) in Roux bottles were incubated 30 days at 25°C in the dark. At the end of incubation, when colony growth had extended all over the surface of the medium, the culture medium was filtered through a 0.2 µm Schleicher & Schull membrane filter BA83. Germination tests were performed by incubating healthy soybean seeds (cv. Amsoy) in plastic petri dishes between blotters. Two experiments were performed: a) seeds were soaked for 5 hours in the culture filtrates and then incubated at 25°C on blotters wet with water; b) seeds were placed on blotters which had previously been moistened with the culture filtrate and incubated under the same conditions. Decreasing effects on seed germination were observed when the second procedure was followed. No irreversible inhibition of germination was seen. On the contrary, there was a delay in the appearance of the first phenotypical stages of germination; that is, most seeds which appeared ungerminated at first

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gradually produced rootlets within 2 to 4 days of incubation. Germination was evaluated by measuring the average length of rootlets at certain intervals. Compared to the sterile distilled water (control), a similar depression was also caused by the culture medium (blank). Compared to the blank, the F strain was seen to be efficient; the activity of the A strain was lower and erratic; B and C strains had either a zero or negligible effect. Culture filtrates of the F strain applied to cuttings of soybean, chickpea, broadbean, pea, bean, and lupin were also capable of inducing leaf yellowing, wilting, and necrosis in various degrees. By comparison, the activity of the A strain was again lower. Using a mineral medium with vitamins added (pH 5) (MV) (Nachmias & Barash (1977), Physiol. Pl. Path. 10: 147-157), the toxicity of culture filtrates apparently increased. After incubation, the A and F strains increased the pH of the Czapek's broth to 7.2 and 7.5 respectively and changed that of the MV to 5.9 and 4.0 respectively. The blanks, HCl-acidified or NaOH-alkalinized to pH values the same as those observed in the culture filtrates, did not show any particular influence either. No remarkable change of effects for either medium was noticed when cultures were incubated for up to 3 months.

In another experiment, filtrates of the A and F strains from 30-day-stationary cultures on Czapek's broth were assayed using 17 cultivars of soybean. The results are given in table 1. Considering the effects on seed germination of all the cultivars as a whole, the F strain was confirmed as efficient whereas the A strain showed no significant effect. Within the limits of this experiment, it appeared that the depressing effect of the F filtrates on single cultivars was significant for 12 of the 17 tested. In comparison, the F strain was also grown in shake culture. After 13 days of incubation, when the mycelium had abundantly grown in the medium, culture filtrates were similarly assayed for their effect on seed germination. All the cultivars as a whole gave similar figures: 5.694 c, 2.203 b and 1.447 a ($P=0.01$; l.s.d.=0.784) for the control, the blank and the F filtrate respectively. However, no particular effect on any one cultivar was observed. It was assumed that the filtrate toxicity in shake culture was broadly lower. Assays on cuttings of the same cultivars (table 2) confirmed the general toxicity of the F strain and the weak or uncertain effect of the A strain in both stationary and shake cultures. At present, results of cultivar by cultivar comparisons of this response with those obtained in the germination test are not possible.

The results of this study can be summarized as follows:

1. Possibly, morphological and physiological discontinuities existing among biotypes of Phomopsis might suggest that particular morphological characteristics are not necessarily related to certain physiological activities reported in this paper. However, even without this connection, the possibility exists that certain strains produce toxic metabolites in culture. If this is found to be true also in vivo, a laboratory seed health test based on the presence of Phomopsis, with all its variability in a sample where different strains are present, would hardly predict the seed performance in the field.

2. The observed detrimental effects of the F strain filtrate do not necessarily mean that phytotoxins are involved (Van Alfen & McMillan 1982, Phytopathology 72:132-135). Whatever substances are responsible, apparently they are produced rather early in culture and increase with colony aging. The importance of such metabolites in the disease syndrome would then be related to the age or growth stage

Table 1 - Length of rootlets (cm) after 4 days of incubation of soybean seeds at 25°C in the presence of culture filtrates of A and F strains of Phomopsis from 30-day-old stationary cultures.

Cultivar	Control	Blank	A Strain	F Strain
Hodgson	6.1	3.6	2.7	0 *
AGRIPO 4124	5.3	2.0	4.2	.1
Wells	4.4	2.6	2.1	.1 *
Coles	1.8	1.6	2.6	.1
Beeson	3.6	1.4	2.0	.1
Verowe	3.1	3.2	2.0	.2 *
Corsoy	6.8	4.9	5.2	.3 *
Amsoy	4.8	4.4	4.5	.3 *
SRF 150/P	5.1	2.5	3.4	.3
Maple Arrow	5.7	2.5	5.5	.4
Kinesoy	7.0	4.7	4.6	.4 *
Williams	4.1	4.1	4.4	.4 *
Evans	7.4	4.9	4.2	.4 *
Swift	6.4	4.0	3.4	.5 *
Steele	6.7	4.1	4.0	.6 *
Weber	8.0	4.1	4.7	.6 *
McCall	6.1	4.8	5.0	.7 *
Between columns				
P=0.01	5.415 c	3.479 b	3.776 b	0.288 a
l.s.d.=0.62				

*Significantly lower than the blank.

Mean of 8 seeds per cultivar.
Control = distilled water;
blank = Czapek's broth.
P=0.01; l.s.d.=2.55

Table 2 - Response of soybean cuttings to culture filtrates of A and F strains of Phomopsis from 30-day-stationary and 13-day-shake cultures, after 48 hours of treatment.

Cultivar	Static Culture				Shake Culture			
	Cntr.	Blk.	A	F	Cntr.	Blk.	A	F
Hodgson	-	±	+	++	-	-	-	++
AGRIPO 4124	-	-	±	++	-	-	-	++
Wells	-	-	-	+	-	-	±	±
Coles	-	-	-	++	-	-	+	+
Beeson	-	-	-	+	-	-	±	+
Verowe	-	-	-	+	-	-	-	±
Corsoy	-	±	±	+	-	-	+	+
Amsoy	-	-	-	++	-	-	-	±
SRF 150/P	-	-	-	++	-	-	-	++
Maple Arrow	-	-	±	++	-	-	-	++
Kinesoy	-	-	-	++	-	-	-	+
Williams	-	-	-	+	-	-	+	++
Evans	-	±	+	++	-	-	-	++
Swift	-	-	-	++	-	-	-	++
Steele	-	-	±	++	-	-	+	+
Weber	-	-	-	++	-	-	-	±
McCall	-	±	_	++	-	-	+	+

- No symptoms

± Slight wilting

\+ Severe wilting

++ Withering and drying

Mean of 5 cuttings per cultivar.
Control = distilled water; blank = Czapek solution.

of the inoculum. This would not be surprising in the soybean-Phomopsis complex, since the greatest amounts of seed deterioration are often related to delayed harvest (wherein environmental conditions exist that are favorable for fungus growth on pods in the field).

3. The mycelial nature of the F strain leads us to believe that it could be Diaporthe phaseolorum var. caulivora. In this case and if phytotoxins are involved, then it would not be a surprise that they are produced by a canker-inducing micro-organism.

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Scanning Electron and Light Microscopy of Phomopsis Anamorphs Commonly Associated with Soybean

Thomas W. Hobbs and
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In Ohio, two types of Phomopsis anamorphs are commonly isolated from soybean plant parts. The first type fits the description of Phomopsis sojae (Lehman) Wehm. (synonym: D. sojae Lehman) (Ann. Mo. Bot. Gard. 10:111-178). The second type differs from P. sojae in several morphological features, some of which were first reported by Kmetz et al. in 1974 (Plant Dis. Rep. 58:978-982). Our study was undertaken to compare cultural morphology of these two Phomopsis types with the morphologies that have been reported for other soybean-derived Phomopsis species.

Materials and Methods

Isolates used in this study were obtained as mycelial cultures from soybean seed from various Ohio counties. The type materials of P. glycines Petrak and P. phaseoli Petch were obtained from Botanische Abteilung, Naturhistorisches Museum, Wien, Austria, and The Herbarium, Royal Botanic Gardens, Kew, Surrey, United Kingdom, respectively.

Cultural morphology and other characteristics of the isolates were compared on plates of potato dextrose agar (acidified to pH 4.5 with 85 percent lactic acid) incubated under intermittent fluorescent light (about 12 h daily) on a laboratory bench (22 to 25°C). Conidiomata were sectioned with a razor blade under a dissecting microscope. For light microscopy, sections were mounted in 15 percent lactic acid and observed using Normarski Interference Contrast. Measurements of 100 alpha conidia were made from each of 10 isolates. Some sections were prepared for scanning electron microscopy by washing with a 0.1 M phosphate buffer (pH 7.0) several times, agitating in 1 percent potassium hydroxide for 30 to 45 min then 1 percent acetic acid for 5 min, and rinsing in 5 to 6 changes of phosphate buffer. Samples were fixed in 1 percent osmium tetroxide (OsO_4) in phosphate buffer overnight at 5°C; then treated 45 min with saturated, aqueous thiocarbohydrazide; and then re-treated for 1 h with OsO_4 at room temperature. After 3 to 4 phosphate buffer rinses, samples were dehydrated in an ethanol series and dried by the critical point method. Specimens were attached to aluminum stubs with silver paint and coated with platinum prior to examination under a scanning electron microscope.

Results and Discussion

Lehman did not describe P. sojae when he published the binomial in 1922 (J. Elisha Mitchell Sci. Soc. 38:13), nor did he refer to either the name Phomopsis sojae or his 1922 paper when he described the anamorphic state of D. sojae in 1923 (loc. cit.). Therefore, prior to the study by Kmetz et al. in 1974 (loc. cit.), most Phomopsis isolates from soybean were recorded as the Phomopsis state of D. phaseolorum var. sojae or just as D. phaseolorum var. sojae. In 1924, however, an anonymous reviewer validated the name Phomopsis sojae by connecting it with Lehman's 1923 description of the Phomopsis state of D. sojae (Rev. Appl. Mycol. 3:377).

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Isolates used in the present study could be divided into two types, based on colony appearance after 2 weeks. Type 1 isolates produced a floccose, ropy mycelium that was initially white but became tan to brown as the culture aged. Isolates of this type formed pulvinate stromata that seldom were larger than 15 mm in diameter. Conidiomata of the type 1 isolates had lenticular locules and seldom developed ostiolate necks longer than 200 µm. Alpha and beta conidia formed within the same conidioma and were borne on simple conidiophores. These isolates fit the description of P. sojae and were always associated with the teleomorph D. phaseolorum var. sojae. These isolates were also similar to those described as D. phaseolorum var. sojae in several other studies.

Type 2 isolates produced dense mycelia that remained mostly white, although some isolates developed greenish-yellow areas. Isolates of this type formed massive, effuse stromata that often extended over the entire bottom of the culture dish. Alpha conidia were produced in conidiomata that had globose locules and long ostiolate necks. Conidiophores frequently were branched. Beta conidia occasionally formed in older stock cultures but were absent from fresh ones. Type 2 isolates never formed perithecia and were identical to Phomopsis sp. sensu Kmetz et al.

Mean alpha-conidial length and width measurements for individual isolates of the two types overlapped, but the overall means were distinct (table 1). Mean alpha-conidial length-to-width ratios were always distinct, although there was some variation among isolates within a type (table 1) and some overlap of the overall ranges for the two types (table 2). Distinguishing characteristics of the two types are summarized in table 2.

Isolates of the second type were also distinct from P. glycines and P. phaseoli Petch, the only other described Phomopsis species from soybean.

Phomopsis glycines was described by Petrak in 1936 (Ann. Mycol. 34:240-241). The type material consisted of two pods on which numerous conidiomata were borne. Conidiomata averaged 198 µm in diameter, had a short or no ostiolate neck, and contained alpha conidia borne on simple conidiophores. Thus, this species is nearly identical to P. sojae.

Phomopsis phaseoli Petch was described in 1922 (Ann. R. Bot. Gard. [Peradeniya]7:311) from soybean stem tissue collected in Ceylon. It is a later homonym of P. phaseoli (Desm.) Sacc. (1915. Nuovo G. Bot. Ital. [Nuova Ser.]22:47), the anamorph of D. phaseolorum var. phaseolorum. Most of the conidiomata of the type material appeared immature. Larger conidiomata bore a short ostiolate neck. Conidiomata averaged only 159 µm in diameter, although Petch reported a diameter of 250 µm. Conidia and conidiophores of this species were not observed, but Petch's measurements of the alpha conidia (3 to 6 by 1.5 to 2 µm) are among the smallest reported for species of Phomopsis isolated from legumes. This species may be an immature specimen of a known species or may represent an entirely new species, and it should be considered a nomen dubium.

Because isolates of type 2 differ in morphology from the other soybean-derived Phomopsis species that have been reported, we feel they represent a new species and have submitted a paper to Mycologia toward that end.

Besides numerous isolates from soybean seed, pod, and stem tissues made during the course of this study, cultures from other locations were examined and

Table 1 - Alpha-conidial length, width, and length-to-width ratios of 2 *Phomopsis* types from soybean.

Type	Isolate	Length	Width	L/W Ratio[a]
1	D167	7.4 C	2.1 E	3.6 A
	D168	7.7 AB	2.2 D	3.5 A
	D94	7.6 B	2.3 C	3.3 B
	D18	7.3 C	2.2 D	3.3 B
	D74	7.8 A	2.4 BC	3.3 B
	Overall mean[b]	7.6	2.2	3.4
2	P68	6.9 D	2.4 B	2.9 CD
	P74	6.9 D	2.6 A	2.7 E
	P43	6.8 D	2.4 B	2.8 DE
	P116	7.3 C	2.4 B	3.0 C
	P32	6.8 D	2.4 BC	2.8 DE
	Overall mean[b]	6.9	2.4	2.9
	FLSD (0.01)	0.2	0.1	0.1

[a]Mean of 100 observations. Means followed by the same letter do not differ significantly according to Duncan's new multiple range test (P=0.05).
[b]Mean of 500 observations.

Table 2 - Comparison of cultural characters for 2 *Phomopsis* types from soybean.

Character	*Phomopsis* type 1	*Phomopsis* type 2
Stromata	Pustulate	Massive, effuse
Ostiolate Necks	<200 µm	200 to 500 µm or longer
Alpha conidia		
Size		
Range	5.6 to 10.3 x 1.5 to 3.4 µm	5.1 to 9.2 x 1.5 to 3.1 µm
Mean[a]	7.6 x 2.2 µm	6.9 x 2.4 µm
L/W ratio		
Range	2.1 to 5.4	1.7 to 4.5
Mean[a]	3.4	2.9
Beta conidia	Abundant	Rare in fresh cultures
Conidiophores	Simple, rarely branched	Simple to frequently branched
Teleomorph	*Diaporthe phaseolorum* var. *sojae*	None

[a]Mean of 500 observations.

identified as similar to type 2 isolates. These included isolates from Illinois (J. B. Sinclair), Iowa (D. C. McGee), Maryland [American type culture collection (ATCC)], and Mississippi (B. L. Keeling). Isolates from other locations identified as P. sojae included those from Great Britain [Commonwealth Mycological Institute (IMI)], Illinois (J. B. Sinclair), Iowa (D. C. McGee), and Maryland (ATCC and M. M. Kulik).

Phomopsis type 2 differs from P. sojae not only morphologically, but ecologically and pathogenically as well. Kmetz et al. reported it to be more prevalent than either of the two D. phaseolorum varieties in immature and mature soybean seed and also in soybean debris. They also reported that it readily rotted inoculated seed but that the other two organisms were mildly or moderately pathogenic to seed.

Differences in alpha-conidial size between the two Phomopsis types can best be detected from isolates grown on artificial media. Although length and width measurement values overlapped, isolates of the two types could be readily differentiated by the mean alpha-conidial sizes and mean alpha-conidial length-to-width ratios. Variation in spore size has been reported to be common among Phyllosticta species, but the length-to-width ratio is fairly constant and useful in distinguishing species in that genus. This ratio also appears to be fairly constant for the two Phomopsis types compared in this study. Further investigations should be conducted to determine if this measure can be used as a reliable tool to differentiate Phomopsis species.

Species in the genus Phomopsis are usually associated with Diaporthe teleomorphs. This is the case for P. sojae. Mature perithecia were found on overwintered stems of soybeans grown in Indiana and Ohio and also in culture. Cultures grown from either single ascospores or alpha conidia usually produce perithecia on artificial media in 4 to 6 weeks, indicating that D. phaseolorum var. sojae is homothallic, as previously reported by Jensen (1983. Mycologia 75:1074-1091). All these cultures have also produced the anamorph P. sojae. A teleomorph for isolates of the type 2 Phomopsis has not been found.

Further investigations should be made to determine the complete host ranges and life and disease cycles of these important soybean pathogens.

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SESSION B. EPIDEMIOLOGY

Bill L. Keeling, Chairman[1]

Epidemiology of the Diaporthe/Phomopsis Complex on Soybeans

James B. Sinclair[2]

The Diaporthe/Phomopsis disease complex of soybean and the causal agents involved have been reviewed in the Compendium of Soybean Diseases (15). The disease complex is endemic in Illinois and reduces germination, vigor, yield, and quality of soybean (14) and other large-seeded legume seeds in the United States and other countries (1, 11, 13, 15). High moisture and temperature increase disease severity (11, 14, 15, 17). Lehman (13) observed that high humidity and warm weather were conducive to seed infection by Phomopsis spp., and Kmetz et al. (12) and Hepperly and Sinclair (8) demonstrated that Phomopsis conidia are disseminated by splashing rain. High levels of seed infection also are associated with delayed harvest (17). There is a positive correlation with the level of disease in the field and seed infection (9). This fact has been used by workers in Illinois, Iowa, Kentucky, Ohio and other States to predict seed infection and whether or not the use of a fungicide(s) would improve soybean seed quality and yields. Information concerned with this practice can be found elsewhere in this publication. We have been studying the epidemiology of Phomopsis seed decay since the early 1970's in Illinois, Brazil, and Puerto Rico. This paper reviews the work in the Department of Plant Pathology, University of Illinois at Urbana-Champaign, on the epidemiology of the Diaporthe/Phomopsis complex of soybeans.

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Epidemiology of Phomopsis Seed Decay

In 1972, 'Amsoy' and 'Wayne' soybean seed lots harvested from northern, central, and southern Illinois were assayed for internally seedborne fungi (16). 'Wayne' seed lots from the central region and south had more Phomopsis spp. than those from the north, with a significant ($P=0.05$) difference between the north and south. There was no significant difference in the occurrence of Phomopsis between regions among 'Amsoy' seed lots.

In a 3-year study of Phomopsis seed decay in Illinois, disease incidence was highest in 1977, lowest in 1976, and intermediate in 1975 (14). A low positive correlation was found between temperature and disease incidence, but no consistent continuum of disease from north to south within the State was apparent. The highest incidence of Phomopsis seed decay occurred along major waterways in the wet years of 1975 and 1977. A high positive correlation was found between disease incidence and rainfall during pod fill, indicating that moisture, rather than temperature or geographic area, is the dominant environmental factor in disease development (fig. 1). Maturity dates of cultivars interacted with weather conditions to affect disease incidence. In our studies, cultivars in maturity group II had the highest level of Phomopsis seed decay. Cultivars used in seed production in Illinois should be grown at latitudes where they will mature late in the season and escape conditions conducive to high incidence of seed decay.

Effect of Cultivar, Pod Height, and Pod Age

The frequency and severity of infections by Phomopsis spp. were inversely related to pod height on soybean plants for the cultivars Chippewa 64, Hark, and Wells (9). Phomopsis spp. seed damage was found

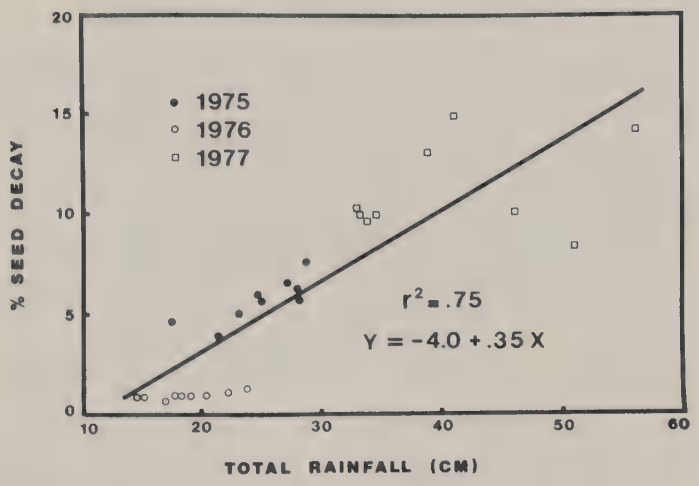

Figure 1.--Relationship between total rainfall during August, September, and October and percentage of Phomopsis seed decay in Illinois. Each point represents one district and year.

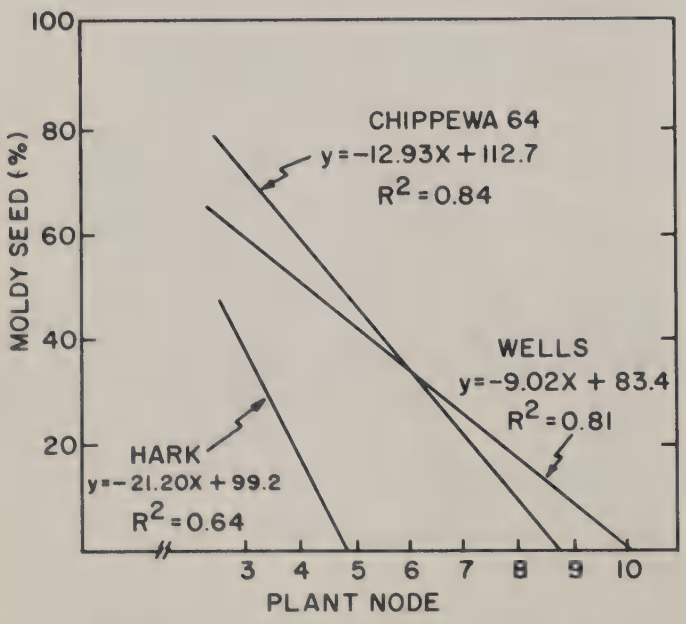

Figure 2.--Linear regression analysis for plant node (independent variable vs. moldy (Phomopsis seed decay) seed percentage (dependent variable) in 3 soybean cultivars.

primarily in the bottom third of the plants, and distribution of damaged seed was skewed heavily to the lowest bearing node (fig. 2). Overall USDA soybean grades were lowered for 'Chippewa 64' and 'Wells' due to seed damage caused by Phomopsis spp. Disease ratings based on pod, seed, and stem symptoms were tested for their association with seed infection rates. The visible symptoms rating for seed was highly associated with rates of seed infection for all cultivars. Pod and stem symptom ratings were highly associated with seed infection rates for 'Chippewa 64' and 'Wells' but not for 'Hark'. Selective harvest of upper plant portions improved seed quality.

Pods with full-sized seeds, which were green (R_6) to brown (R_8), were detached from greenhouse plants, surface-disinfected, and inoculated with Phomopsis spp. (10). After 1 week at 95 percent relative humidity and 25°C, wound-inoculated 'Corsoy' pods (R_6) had higher frequencies of pod lesions and seed infection and reduced germination than surface-inoculated pods. Stage of pod senescence was critical for rapid seed colonization. Low levels of seed infection were found in green 'Harosoy' pods (R_6), whereas high levels were found in yellow (R_7) and brown (R_8) pods. Increasing the period of pod incubation increased the rate of seed infection, regardless of pod maturity. Lesions on green pods were centered about trichomes. Pubescent cultivars Corsoy, Hark, and Harosoy developed more pod lesions than the sparsely pubescent 'Chippewa'. Disease assessment of inoculated intact plants and inoculated, detached pods of 'Hark', 'Rampage', 'Wells', and 'Williams' showed that results from both methods were closely correlated and that cultivars Hark and Williams were less susceptible to Phomopsis spp. than 'Rampage' and 'Wells'.

Interaction Between Phomopsis spp. and Cercospora kikuchii

Seeds from similar field plots planted to four soybean cultivars in Illinois and Puerto Rico were assayed for mycoflora (11). The predominant fungus recovered was Phomopsis spp. from Illinois seeds and Cercospora kikuchii from Puerto Rico seeds. Purple-stained seed had higher germination at normal harvest than nonstained seed. C. kikuchii from Puerto Rico-grown seeds was antagonistic to seedborne Fusarium spp. and Phomopsis sp., which were recovered six and three times more often, respectively, from nonstained than from purple-stained seeds. C. kikuchii and Phomopsis sp. were not antagonistic in Illinois seeds except when the incidence of C. kikuchii exceeded 10 percent. Recoveries of Fusarium and Phomopsis spp. increased and germination and recoveries of C. kikuchii decreased when harvest was delayed in Puerto Rico. Multiple regression equations related the occurrence of C. kikuchii, Fusarium, and Phomopsis spp. to reduced soybean seed germination in Puerto Rico. In Illinois, variation in the incidence of Phomopsis explained most of the variation in germination.

'Amsoy 71' soybean seeds with seed coats showing differing amounts of purple stain caused by C. kikuchii were placed on potato-dextrose agar (PDA), moist cellulose pads, or in sand (19). The greater the amount of the seed coat showing purple stain, the greater was the reduction in germination. Germination always was highest on PDA and lowest on cellulose pads. Stunted and low vigor seedlings resulted from seeds having 50 percent or more of the surface stained. No antagonism was observed between C. kikuchii and Phomopsis sp. in dual culture on PDA. However, culture filtrates from C. kikuchii stimulated the growth of Phomopsis sp., causal fungus of soybean seed decay (fig. 3). A linear relationship was recorded between the concentration of this culture filtrate and colony size of Phomopsis sp. growing on water agar.

Cercosporin extracted from purple-stained seed coats did not inhibit growth of Phomopsis sp. at concentration as high as 1,000 µg/mL. Nutritional depletion and/or competition for space is suggested to explain the low incidence of Phomopsis sp. in soybean seeds colonized by C. kikuchii.

Interaction Between Phomopsis spp. and Soybean Mosaic Virus

Twenty-five soybean lines were grown in the field and either inoculated at the primary leaf stage with the Illinois severe isolate of soybean mosaic virus (SMV) or noninoculated (6). In 20 lines, SMV inoculation reduced germination and increased seedborne incidence of Phomopsis

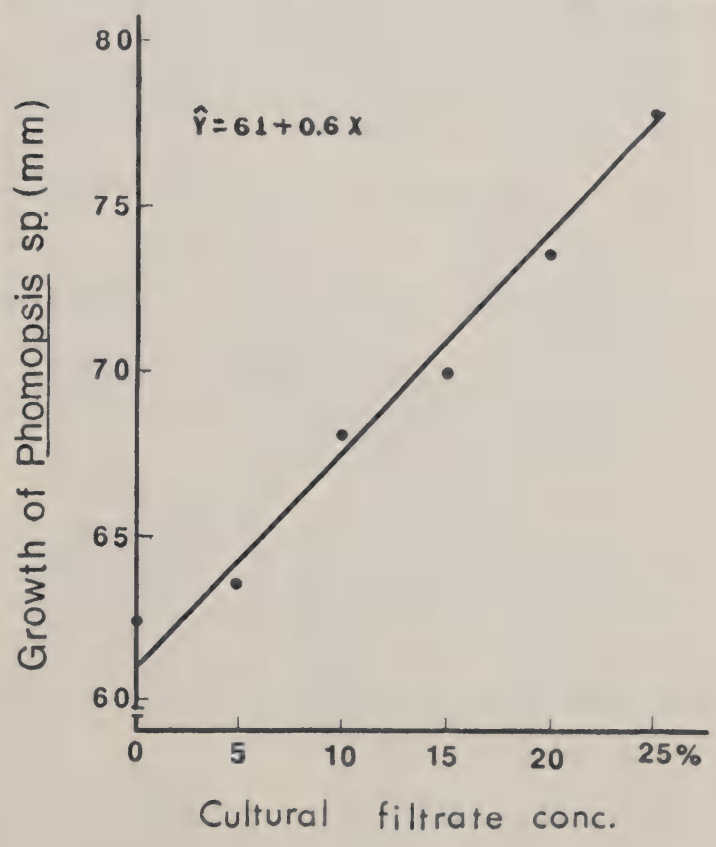

Figure 3.--Relationship between the concentration of a culture filtrate from Cercospora kikuchii and colony size of Phomopsis sp. on 2 percent water agar after 7 days at 25°C.

spp. Inoculation of 'Williams' soybeans with SMV before or during flowering reduced germination and increased the incidence of Phomopsis spp. seed infection. Inoculation with SMV during pod development neither reduced germination nor increased seed infection by Phomopsis spp. Regardless of the date of inoculation, 'Williams' soybeans inoculated with SMV always yielded less than noninoculated plants. 'A. K. Harrow' and 'Mansoy' soybean plants grown in controlled-environment chambers were inoculated with SMV or Phomopsis spp. alone or in combination. The production of seeds with symptoms of Phomopsis spp. infection (moldy seeds) required both SMV and Phomopsis spp. inoculation. SMV alone did not reduce seed germination or lead to production of damaged seed. When plants inoculated with SMV showed increased susceptibility to Phomopsis spp., seed germination was reduced and damaged seed production was increased.

Interaction Between Phomopsis spp. and a Nonpathogen

Chaetomium cupreum was isolated from three soybean seed lots of two cultivars grown at three locations in Illinois in 1979 (18). This was the first report of C. cupreum in soybean seeds. In dual cultures, zones of inhibition developed between C. cupreum and Fusarium sp., Macrophomina phaseolina, Phomopsis sp., and Rhizoctonia solani. The growth of C. kikuchii, Colletotrichum truncatum, Fusarium sp., M. phaseolina, Phomopsis sp., and R. solani but not of Cercospora sojina or Gliocladium roseum was inhibited on water agar that had been mixed with the culture filtrate of C. cupreum and autoclaved. Ethyl ether soluble fractions of the culture filtrate of C. cupreum inhibited the growth of all fungi mentioned and of Alternaria sp. and delayed germination of soybean seeds. The fraction had absorption maxima at 230, 250, 280, 290, and 505 nm in water.

Alternative Hosts

Phomopsis spp. was isolated from Amaranthus spinosus, Leonotis nepetaefolia, and Leonurus sibiricus, common weeds in soybeans grown in southern Brazil (2). The latter two hosts were symptomless carriers of Phomopsis spp. Pycnidia produced by cultures of Phomopsis spp. isolated from the three weed hosts gave rise to alpha and beta conidia typical of Diaporthe phaseolorum. The Phomopsis spp. isolates from L. nepetaefolia and L. sibiricus, but not from A. spinosus, reduced seed germination, radicle length, and emergence of 'UFV$_1$' soybeans but not of 'Rico 23' common bean (Phaseolus vulgaris) grown in infested sand or soil. These weed isolates colonized soybean stems and produced pycnidia. Isolates from A. spinosus did not produce pycnidia on common bean stems.

Three pathogens of soybean, Phomopsis spp., Colletotrichum truncatum, and C. gloeosporioides (Glomerella cingulata) were isolated from velvetleaf, a common weed in Illinois soybean fields (7). A lethal stem canker induced by Phomopsis sp. developed in 1 to 2 percent of velvetleaf plants examined in Champaign County, and the pathogen was recovered from 25 to 65 percent of the stem sections when plants were $\geq$ 4 weeks old. Recoveries of C. gloeosporioides and C. truncatum ranged from 0 to 35 percent and 0 to 85 percent respectively. Inoculation of 'Amsoy 71' soybean seedlings with C. gloeosporioides induced leaf cupping and veinal necrosis on expanding leaves. Velvetleaf isolates of C. truncatum and Phomopsis spp. usually were more virulent on soybean pods than were soybean isolates. Isolates of C. gloeosporioides from soybean and velvetleaf were avirulent on soybean pods. Inoculation of soybean pods with Colletotrichum spp. reduced Phomopsis sp. in seed. Velvetleaf isolates of C. truncatum and Phomopsis sp.

had a greater growth rate than soybean isolates on soybean pods and seed extract agar, and all isolates regardless of host source grew more rapidly on velvetleaf stem extract agar.

Use of Desiccants to Detect Latent Infection

Soybean stems and pods were surface-disinfested, then immersed in a solution of commercial paraquat and incubated for 4 days under continuous light at 25°C (4). A greater number of lesions with fruiting structures and conidia of Cercospora kikuchii, Fusarium spp., and Phomopsis spp. were formed on plant parts immersed in paraquat than on nonimmersed tissues. There was no increase in the occurrence of Alternaria spp. Nontreated plant parts incubated for an additional 6 days failed to develop lesions in numbers equal to those of the paraquat-treated tissues. Field application of paraquat resulted in greater numbers of acervuli of Colletotrichum truncatum and pycnidia of Phomopsis spp. on stems of 'Bonus' and 'Wells' soybeans than on nonsprayed plants. Soybean cultivars UFV_1 and UFV_2 grown at Florestal and Viçosa, Brazil, and cultivars Bonus and Wells grown at Urbana, IL, were sprayed at growth stages $R_{5.5-6}$ (full pod) or R_7 (physiologic maturity) with one of three desiccant-type, nonselective herbicides: glyphosate, paraquat, or sodium chlorate:sodium borate (50:50). The stems of all paraquat-sprayed plants had optimum development of fruiting structures of Colletotrichum truncatum and Phomopsis spp. as much as 3 weeks earlier than nonsprayed plants (1). Glyphosate-sprayed 'Bonus' and 'UFV_1' plants gave similar results. No differences were noted between nonsprayed plants and plants sprayed with sodium chlorate:sodium borate. Parallel increases in the development of fruiting structures with time were recorded on stems of nonsprayed and sprayed plants. Treatment, time of treatment, and location influenced the development of the fruiting structures of the test fungi. Desiccation of plants by paraquat significantly ($P=0.05$) reduced seed weight and germination at all locations and increased the incidence of Alternaria and Phomopsis spp. at Urbana (3). Analysis of the combined data from the Brazilian locations showed a significant decrease in seed germination for all treatments except paraquat sprayed on the cultivar UFV_2 at R_7 and sodium chlorate:sodium borate sprayed on the cultivar UFV_1 at R_7. Herbicide application at R_7 did not result in consistent increases in seedborne Fusarium or Phomopsis spp. at any Brazilian location. Rainfall and temperature at seed maturation were more important variables in pod-to-seed infection by these fungi than increased rates of tissue senescence caused by the desiccants.

References

1. Cerkauskas, R. F., O. D. Dhingra, 1983 and J. B. Sinclair. Effect of three desiccant-type herbicides on fruiting structures of Colletotrichum truncatum and Phomopsis spp. on soybean stems. Plant Disease 67:620-622.

2. Cerkauskas, R. F., O. D. Dhingra, 1983 J. B. Sinclair, and G. Asmus. Amaranthus spinosus, Leonotis nepetaefolia, and Leonurus sibiricus: new hosts of Phomopsis spp. in Brazil. Plant Disease 67:821-824.

3. Cerkauskas, R. F., O. D. Dhingra, 1982 J. B. Sinclair and S. R. Foor. Effect of three desiccant herbicides on soybean (Glycine max) seed quality. Weed Sci. 30:484-490.

4. Cerkauskas, R. F., and J. B. Sinclair. 1980 Use of paraquat to aid detection of fungi in soybean tissues. Phytopathology 70:1036-1038.

5. Foor, S. R., F. D. Tenne, and 1976 J. B. Sinclair. Occurrence of seedborne microorganisms and germination in culture for determining seed health in soybeans. Plant Dis. Rep. 60:970-973.

6. Hepperly, P. R., G. R. Bowers, 1979 Jr., J. B. Sinclair, and R. M. Goodman. Predisposition to seed infection by Phomopsis sojae in soybean plants infected by soybean mosaic virus. Phytopathology 69:846-848.

7. Hepperly, P. R., B. L. Kirkpatrick, 1980 and J. B. Sinclair. Abutilon theophrasii: wild host for three fungal parasites of soybean. Phytopathology 70:307-310.

8. Hepperly, P. R., and J. B. Sinclair. 1978 Distribution of Phomopsis-infected seeds on field-grown soybeans. (Abstr.) Phytopathol. News 12:88.

9. Hepperly, P. R., and J. B. Sinclair. 1980 Associations of plant symptoms and pod position with Phomopsis sojae seed infection and damage in soybean. Crop Sci. 20:379-381.

10. Hepperly, P. R., and J. B. Sinclair. 1980 Detached pods for studies of Phomopsis sojae pods and seed colonization. J. Agr. Univ. Puerto Rico 64:330-337.

11. Hepperly, P. R., and J. B. Sinclair. 1981 Relationships among Cercospora kikuchii, other seed mycoflora and germination of soybeans in Puerto Rico and Illinois. Plant Disease 65:130-132.

12. Kmetz, K. T., Ellett, C. W., and 1979 A. F. Schmitthenner. Soybean seed decay: sources of inoculum and nature of infection. Phytopathology 69:789-801.

13. Lehman, S. G. 1923 Pod and stem blight of soybean. Ann. Mo. Bot. Gard. 10:111-169.

14. Shortt, B. J., A. P. Grybauskas, 1981 F. D. Tenne, and J. B. Sinclair. Epidemiology of Phomopsis seed decay of soybean in Illinois. Plant Disease 65:62-64.

15. Sinclair, J. B. 1982 Compendium of soybean diseases. Second edition. 104 p. Amer. Phytopathol. Soc., St. Paul, MN.

16. Tenne, F. D., C. Prasartsee, 1974 C. C. Machado, and J. B.Sinclair. Variation in germination and seedborne pathogens among soybean seedlots from three regions in Illinois. Plant Dis. Rep. 58:411-413.

17. Wilcox, J. R., F. A. Laviolette, and 1974 K. L. Athow. Deterioration of soybean seed quality associated with delayed harvest. Plant Dis. Rep. 58:130-133.

18. Yeh, C. C., and J. B. Sinclair. 1980 Effect of Chaetomium cupreum on seed germination and antagonism to other seedborne fungi of soybean. Plant Disease 64:468-470.

19. Yeh, C. C., and J. B. Sinclair. 1982 Effect of Cercospora kikuchii on soybean seed germination and its interaction with Phomopsis sp. Phytopath. Z. 105:265-270.

Effects of Soil Moisture on Seedborne *Phomopsis* sp. and Soybean Seedling Performance

Mark L. Gleason and Richard S. Ferriss[1]

Introduction

Pod and stem blight (PSB) is the most serious disease affecting soybean seed quality in Kentucky. The major cause of losses due to PSB is poor germination of infected seeds; the percentage of seeds infected with PSB is often negatively correlated with germination (Ellis et al. 1974, Hepperly and Sinclair 1981, Nicholson et al. 1972, Paschal and Ellis 1978). When PSB fungi become seedborne, the disease is sometime termed *Phomopsis* seed decay because *Phomopsis* sp. is more abundant in most infected seedlots than other PSB fungi and appears to be more pathogenic (Kmetz et al. 1974, 1978).

The ability of most commonly used laboratory tests to predict field performance of PSB-infected seedlots is quite limited. Despite a generally inverse relationship between percent PSB infection of seeds and germination, vigor, or emergence, the relationship is inconsistent in field trials (Kulik and Schoen 1981).

One factor that may hinder success in predicting field performance of PSB-infected seeds is our lack of understanding of how the soil environment interacts with infected seeds. A seed placed in soil is subject to an array of physical and biotic interactions. Soil moisture is among the most critical of the physical variables; however, its interactions with seedborne pathogens are not well known. Kulik and Schoen (1981) observed no correlation between soil moisture level and field emergence of PSB-infected soybeans, but they tested only two moisture levels and did not quantify these. In a greenhouse experiment, Ferriss et al. (1983) noted that a PSB-infected seed lot had much lower percent establishment in certain dry (-3 to -12 bars) and saturated soil treatments than either the same diseased lot in moderately moist soil treatment (-.02 to -.04 bars) or a healthy seed lot. The PSB-infected lot performed poorly in pasteurized as well as natural soil, suggesting that the seedborne microflora played a major role in limiting seedling development.

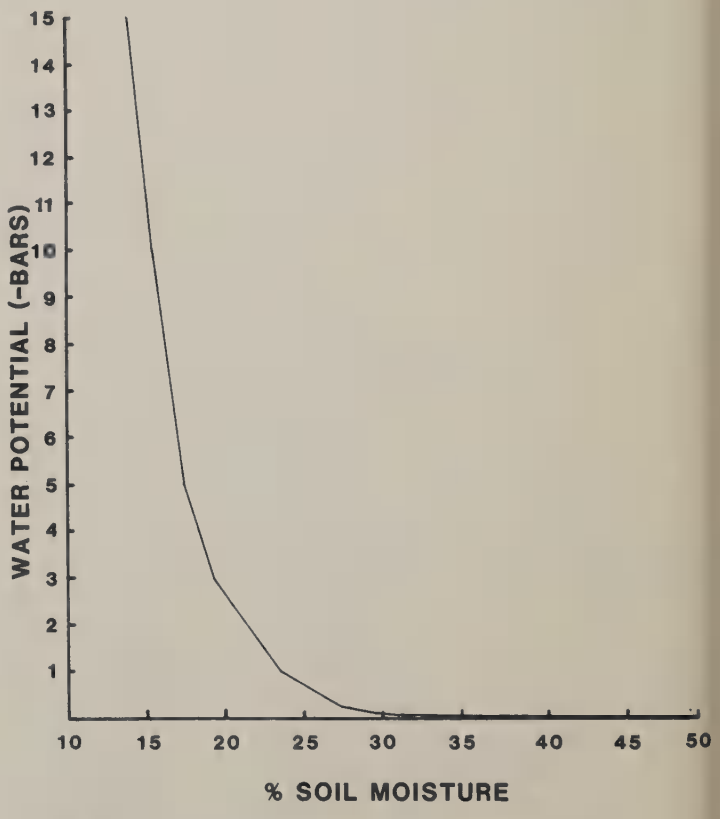

Figure 1.--Composite moisture-characteristic curve for the Maury silt loam soil used in all experiments.

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The present work was undertaken 1) to assess the possible influence of soil moisture on performance of PSB-infected soybean seed lots, 2) to more clearly define the role of seedborne Phomopsis sp. in this interaction, and 3) to determine how soilborne pathogens affect the relationship between soil moisture and seedborne disease.

Materials and Methods

A moisture-characteristic curve was determined by pressure plate and tension plate methods for the Maury silt loam soil (from Spindletop Research Farm of the University of Kentucky) used in the experiments (fig. 1). A pressure plate was used from -1 bar to -15 bars and a tension plate apparatus was used over the range -0.1 bar to saturation. Water potential for soil drier than -15 bars was estimated by the filter paper method of Fawcett and Collis-George (1967). Soil moisture was adjusted to levels corresponding to chosen soil water potentials by air-drying or by adding water.

Shredded soil was pasteurized by microwaving in 4-kg amounts for 425 seconds (Ferriss 1984), a treatment sufficient to greatly reduce populations of many soilborne plant pathogens (table 1).

Seed lots (six of cv. Williams, one of cv. Cumberland) were assayed prior to the experiments for percent infection by PSB fungi and other micro-organisms on potato dextrose agar (PDA) amended with streptomycin sulfate and chlortetracycline (table 2). Phomopsis sp. constituted more

Table 1 - Microbial populations[a] in natural and pasteurized soil[b] used in the experiment shown in figure 2.

	Natural soil	Pasteurized soil[c]
Fusarium spp. (x 10^2)	130	2
Pythium spp.	2,000	1
Rhizoctonia spp.	.17	.02
Total fungi (x 10^3)	22.0	2.3
Total bacteria (x 10^7)	17.9	6.5
Total actinomycetes (x 10^5)	5.3	10.0

[a]Mean number of colonies per gram dry soil; n=3.
[b]Soil moisture: Natural soil = 17.7 percent, pasteurized soil = 17.2 percent.
[c]Assayed 3 weeks after microwave oven treatment.

Table 2A - Quality and vigor indices of seedlots[a] used in experiments.

Seedlot	Percentage				Accelerated aging[b]	Conductivity[c]	Cold test[d]
	3-day germination	Standard germination	Abnormal	Dead			
1	ND[e]	ND	ND	ND	ND	ND	ND
2	55	66	28	7	47	81	49
3	46	55	33	12	62	91	37
4	68	83	12	5	3	75	12
5	79	92	8	1	86	49	74
6	35	42	33	25	45	ND	26
7	65	71	22	7	56	ND	ND

[a]Seedlots 1 through 6 are cv. Williams, seedlot 7 is cv. Cumberland.
[b]Standard germination percentage after 72 h at 41°C and 100 percent relative humidity followed by 5 days at 25°C.
[c]µmho/g seed wet weight.
[d]Standard germination after 7 days' incubation in moist natural soil at 10°C, then 5 days at 25°C.
[e]ND = not determined.

Table 2B - Frequency of occurrence[a] of certain seedborne micro-organisms in seedlots[b] used in experiments.

Seed-lot	Tissue	*Phomopsis* sp. and DPS[c]	*Fusarium*	*Cercospora*	*Alternaria*	*Aspergillus*	*Penicillium*	Bacteria	Not infected
1	Cotyledons	65	ND[d]	ND	ND	ND	ND	ND	ND
	Seedcoats	81	ND	ND	ND	ND	ND	ND	ND
2	Cotyledons	18	1	20	10	0	2	10	29
	Seedcoats	29	2	42	14	0	4	4	2
3	Cotyledons	18	1	10	7	1	3	2	50
	Seedcoats	30	0	33	20	1	2	2	18
4	Cotyledons	0	2	0	26	2	4	9	43
	Seedcoats	0	2	1	44	6	10	7	6
5	Cotyledons	1	2	0	18	1	1	4	66
	Seedcoats	1	3	9	42	9	8	14	2
6	Cotyledons	24	1	2	0	0	0	0	70
	Seedcoats	42	0	18	8	1	0	1	26
7	Cotyledons	20	0	4	4	0	0	0	63
	Seedcoats	45	0	19	8	1	5	0	0

[a]Percentage of cotyledons or seedcoats infected.
[b]Seedlots 1 through 6 are cv. Williams, seedlot 7 is cv. Cumberland.
[c]DPS = *Diaporthe phaseolorum* var. *sojae*.
[d]ND = Not determined.

than 95 percent of PSB colonies in all seedlots.

Seed treatments included benomyl (Benlate 50W), carboxin-thiram (Vitavax 200), and a check treatment in which only deionized water was added to seeds.

In growth chamber and greenhouse experiments, seeds were incubated in soil in plastic trays for 3 days at soil moistures corresponding to chosen values of soil water potential; treatments ranged from approximately 59 percent soil H_2O (saturation) to 9 percent soil water content (-60 bars). After 3 days, soil moisture was equalized in all treatments and maintained near optimum moisture for seed germination and development from day 3 to day 21 by daily watering. Emergence (elevation of the seedling above the plane of the soil surface) and establishment (full opening of a true leaf) were recorded at regular intervals from day 3 to day 21.

In one growth chamber trial, half the seeds in each treatment were removed after 3 days, washed free of soil, and surface sterilized 30 seconds in 10 percent Clorox; then, the seedcoats and cotyledons were plated separately on PDA containing antibiotics. Percent occurrence of seedborne microflora was determined after 7 days' incubation at 21 to 24°C.

Air temperatures ranged from 22 to 25°C in growth chamber trials and 21 to 27°C in the greenhouse.

Results and Discussion

Establishment from a heavily Phomopsis-infected seed lot (lot 6, table 2) and a lightly Phomopsis-infected lot (lot 5, table 2) with benomyl, carboxin-thiram, or check seed treatments was determined in pasteurized and natural soil in a greenhouse experiment (fig. 2). In pasteurized soil, establishment from high-disease check seeds was highest at intermediate soil moisture treatments (23 to 36 percent soil water content) (fig. 2A). Both fungicide treatments increased establishment in treatments of 36 percent soil moisture and drier, with benomyl giving the largest improvement; in

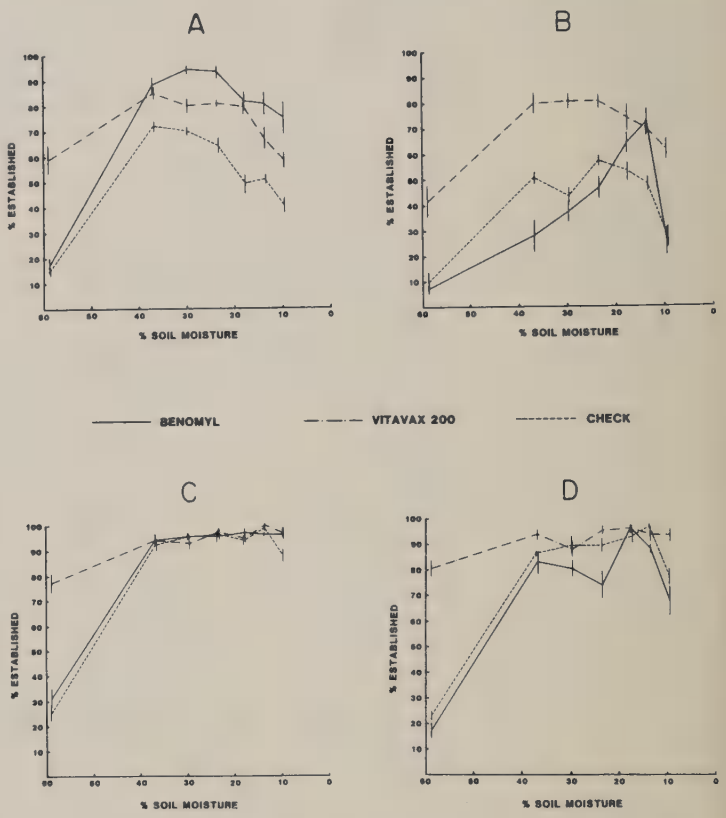

Figure 2.--Percent establishment 21 days after planting a heavily Phomopsis-infected seed lot (lot 6, table 2) and a lightly Phomopsis-infected lot (lot 5) in a greenhouse experiment. Seeds were incubated for 3 days at soil moistures shown on the abscissa, after which all treatments were maintained at soil moistures near the optimum for seed germination and development: (A) the high-Phomopsis lot incubated in pasteurized soil, (B) the high-Phomopsis lot incubated in natural soil, (C) the low-Phomopsis lot incubated in pasteurized soil, (D) the low-Phomopsis lot incubated in natural soil.

contrast, carboxin-thiram was clearly superior in the saturated treatment.

When the high-Phomopsis lot was incubated in natural soil, establishment was less than in pasteurized soil for all soil moisture treatments; the largest differences were for benomyl and check treatments at intermediate soil moisture regimes. Carboxin-thiram gave the best protection over the entire moisture treatment range.

The low-Phomopsis seed lot showed much higher establishment in the drier treatments than did the high-Phomopsis lot and relatively small differences among seed treatments except in saturated soil (figs. 2C and 2D).

Losses were most severe in the saturated soil regime for all seedlot and soil treatment combinations. Treatment with carboxin-thiram sharply improved establishment.

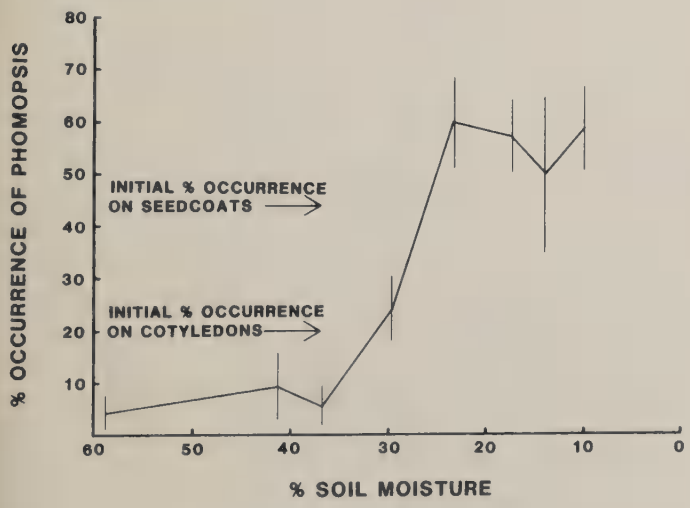

Figure 3.--Percent occurrence of Phomopsis sp. on cotyledons following a 3-day incubation of a heavily Phomopsis-infected seed lot (lot 7, table 2) in pasteurized soil. Initial (preplant) percent occurrence of Phomopsis on seedcoats and cotyledons is indicated by arrows.

In sum, these findings suggest that--

1) Losses induced by seedborne micro-organisms are greatest in relatively dry soil,

2) Soilborne pathogens damage diseased seeds most under moist to wet soil conditions, and

3) Carboxin-thiram is superior to benomyl as a seed treatment under most soil moisture conditions.

After a 3-day growth chamber incubation of a diseased seed lot (lot 7, table 2) in pasteurized soil, the percentage of cotyledons infected by Phomopsis sp. rose sharply in soils of 23 percent water content or drier but decreased in soil of 36 percent moisture or wetter (fig. 3). This response indicates that Phomopsis survived poorly in moist to wet conditions

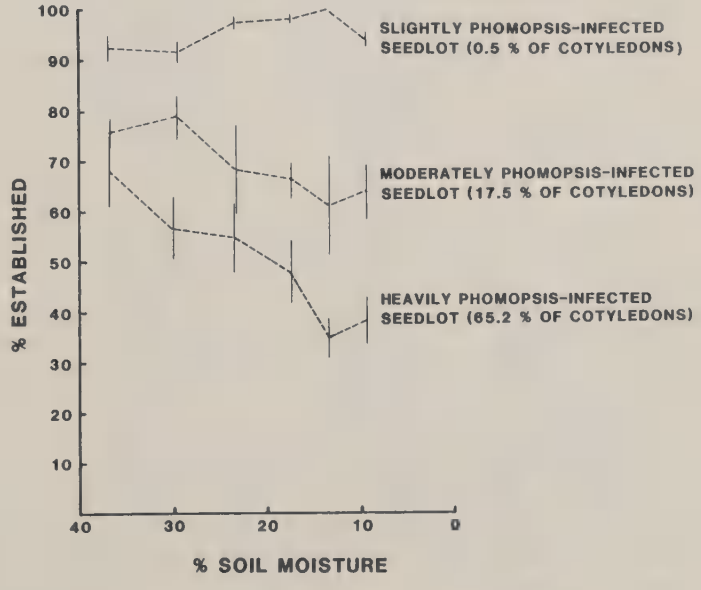

Figure 4.--Percent establishment, at 21 days after planting in pasteurized soil, of 3 seed lots (lots 1, 3 and 5, table 2) differing in percentage of cotyledons initially infected by Phomopsis sp. Soil moistures shown on the abscissa were maintained for 3 days following planting.

Table 3 - Correlation coefficients (r) of percent occurrence of major fungal pathogens initially colonizing 5 Williams seedlots (lots 2 through 6, table 2) with 21-day emergence and establishment in 2 dry pasteurized soil treatments.[a]

	Phomopsis spp.		*Fusarium* spp.		*Cercospora kikuchii*		*Alternaria* spp.	
	Seedcoat	Cotyledon	Seedcoat	Cotyledon	Seedcoat	Cotyledon	Seedcoat	Cotyledon
14% Soil Moisture								
Emergence	-0.97**[b]	-0.97**	NS	0.97**	NS	NS	0.98**	0.89*
Establishment	- .99**	- .98**	NS	.95*	NS	NS	.98**	.91*
10% Soil Moisture								
Emergence	- .96**	- .96**	NS	.96**	NS	NS	.96**	NS
Establishment	- .97**	- .97**	NS	.97**	NS	NS	.97**	.88*

[a]Soil maintained at the indicated moisture levels for 3 days, then equalized to levels optimal for seedling development.

[b]NS=P>0.05; *=0.05>P>.01; **=0.01>0.001; N=5.

but grew rapidly from seedcoats to cotyledons in drier soil. Establishment of seedlings after a 3-day incubation in pasteurized soil was inversely proportional to the percentage of Phomopsis-infected cotyledons for three seed lots (lots 1, 3, and 5) (fig. 4). More heavily Phomopsis-infected lots incurred greater losses, and this difference widened with increasingly dry incubation conditions.

Among the major seedborne pathogens in five 'Williams' seed lots (lots 2, 3, 4, 5, and 6), Phomopsis sp. was the only one whose frequency of occurrence showed significant negative correlations with emergence (EM) or establishment (ES) in dry-soil treatments (table 3). That is, only in the case of Phomopsis was a higher percentage of infected seeds associated with poorer seedlot performance. Significant positive correlations of EM/ES with seedborne Fusarium spp. and Alternaria spp. suggest the possibility that these fungi acted as antagonists of Phomopsis sp.

Taken together, the above evidence implicates Phomopsis sp. as the major seedborne contributor to preemergence losses in dry soil. This finding suggests that performance of Phomopsis-infected seed lots can be predicted on the basis of soil moisture. Leach (1947) incubated seeds of several crop species at several temperatures in soil artificially infested with various pathogenic fungi. He concluded that, for each particular host-pathogen combination, the effect of temperature on emergence is proportional to the ratio of seedling emergence rate (in the absence of the pathogen) to pathogen growth rate at that temperature. Leach's concept can be extended from soilborne pathogens and temperature to the present case involving Phomopsis sp. and soil moisture.

The radial growth rate of Phomopsis was measured on PDA osmotically adjusted to chosen water potentials with sucrose (J. C. Rupe and R. S. Ferriss, elsewhere in these proceedings), and seedling emergence rate was derived as the inverse of the time required for 50 percent of seedlings to emerge from a lightly infected seed lot when 3-day controlled soil moisture treatments were followed by optimization of soil moisture content (fig. 5A). The ratio of host emergence rate to pathogen growth rate closely predicted mean final emergence of two

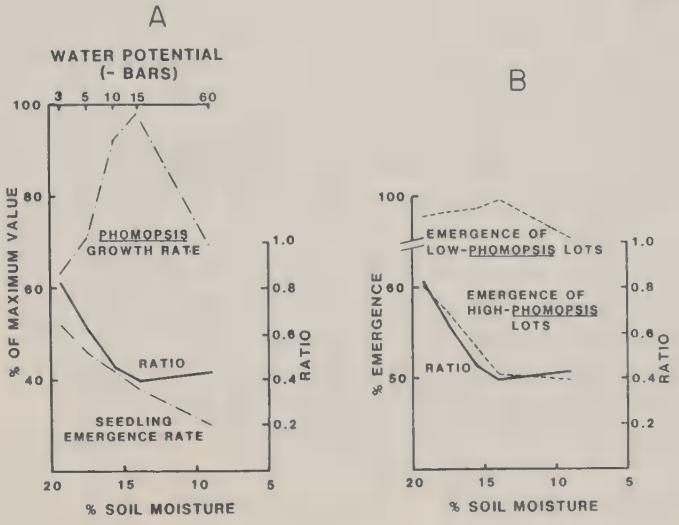

Figure 5.--(A) Phomopsis radial growth rate at 25°C on PDA osmotically adjusted to selected water potentials with sucrose, seedling emergence rate at soil moisture treatments corresponding to these water potentials, and the ratio of seedling emergence rate to pathogen growth rate. Soil moistures indicated on the abscissa were maintained for 3 days after planting, followed by 18 days of soil moistures near the optimum for seedling development. (B) The same ratio derived in (A) in comparison to mean 21-day emergence of 2 high-Phomopsis seed lots (lots 1 and 6, table 2) and 2 low-Phomopsis seed lots (lots 4 and 5).

high-Phomopsis seed lots (lots 1 and 6) in pasteurized soil over a range of soil moisture treatments from 19 percent to 9 percent soil water, but did not predict mean final emergence of two low-Phomopsis seed lots (lots 4 and 5) over the same soil moisture range (fig. 5B). Despite several limitations of this analysis (the water potential value, -60 bars, for the driest soil treatment, 9 percent soil water, is an approximation; Phomopsis growth rate on PDA was measured in response to osmotic water potential rather than the matric potential that dominates in most soils; and soil water potential is a less direct influence on seedborne pathogen growth than seed water potential), it reinforces Leach's (1947) contention that seed performance is controlled by a race between host and pathogen growth rates, mediated by the physical environment. This predictive method has the potential to help clarify important seed-environment relationships and, therefore, should aid in rational selection of seed treatments and improvement of seed quality tests.

References

Ellis, M. A., M. B. Ilyas, F. D. Tenne, and others. 1974. Effect of foliar application of benomyl on internally seedborne fungi and pod and stem blight in soybean. Pl. Dis. Rep. 58:760-763.

Fawcett, R. G. and N. Collis-George. 1967. A filter paper method for determining the moisture characteristics of soil. Aust. J. Exp. Agr. Anim. Husb. 7:162-167.

Ferriss, R. S. 1984. Effects of microwave oven treatment on micro-organisms in soil. Phytopathology 74:121-126.

Ferriss, R. S., R. E. Stuckey, and M. R. Siegel. 1983. Effects of soil source, soil moisture, seed quality and seed treatment on soybean emergence in the greenhouse. Phytopathology 73:812 (abstr.).

Hepperly, P. R. and J. B. Sinclair. 1981. Relationships among Cercospora kikuchii, other seed mycoflora and germination of soybean seeds in Puerto Rico and Illinois. Plant Disease 65:130-132.

Kmetz, K. A., C. W. Ellett, and A. F. Schmitthenner. 1974. Isolation of seedborne Diaporthe phaseolorum var. sojae and Phomopsis from immature soybean plants. Pl. Dis. Rep. 58:978-982.

Kmetz, K. A., A. F. Schmitthenner, and C. W. Ellett. 1978. Soybean seed decay: prevalence of infection and symptom expression caused by Phomopsis sp., Diaporthe phaseolorum var. sojae and D. phaseolorum var. caulivora. Phytopath. 68:836-840.

Kulik, M. M. and J. F. Schoen. 1981. Effect of seedborne Diaporthe phaseolorum var. sojae on germination, emergence and vigor of soybean seedlings. Phytopath. 71:544-547.

Leach, L. D. 1947. Growth rates of host and pathogen as factors determining the severity of pre-emergence damping off. J. Agric. Res. 75:161-179.

Nicholson, J. F., O. D. Dhingra, and
1972 J. B. Sinclair. Internal seedborne nature of *Sclerotinia sclerotiorum* and *Phomopsis* sp. and their effects on soybean seed quality. Mycopath. Mycol. Appl. 50:179-182. [Corrected title: *Sclerotinia sclerotiorum* should be read as *Diaporthe phaseolorum* var. *sojae*.]

Paschal, E. H. and M. A. Ellis.
1978 Variations in seed quality characteristics of tropically grown soybean. Crop Sci. 18:837-840.

Environmental Factors Affecting Movement of *Phomopsis* spp. from Soybean Pods to Seeds

A. Balducchi and D. C. McGee[1]

Phomopsis seed decay causes reduced germination and vigor of soybean seeds. Control can be achieved by applying fungicides to the growing seed crop. The disease often is not severe enough, however, to justify fungicide treatment. A predictive method using *Phomopsis* pod infection at the R6 growth stage to indicate severity of seed infection can identify fields that should be sprayed. A limitation of this method is that seed infection is also influenced by weather conditions between the time that pod infection is measured and harvest maturity. If the conditions that favored seed infection were known, tolerances of pod infection levels indicating the need for fungicide application could be adjusted for geographical regions, according to the probability of these conditions occurring. The objective of this study was to define these conditions.

Effects of relative humidity and temperature on seed infection were examined, under laboratory conditions, in pods detached from plants at the R8 growth stage. Pods were placed on metal racks supported about 1 cm above blotters in plastic boxes. These were exposed either to 100 percent relative humidity by wetting the blotters and enclosing the boxes in plastic bags or to ambient relative humidity (40 to 60 percent) in incubators by leaving the blotters dry and not enclosing the boxes. In one experiment, pods detached from greenhouse-grown plants were immersed in a spore suspension of *Phomopsis* sp. and then exposed to different sequences of days at either 100 percent or 40 to 60 percent relative humidity. *Phomopsis* seed infection was measured at the end of each treatment period by plating seeds on potato dextrose agar. All treatments were carried out in an incubator held at 25°C.

These data show that at least three continuous days of 100 percent relative humidity were needed for extensive seed infection to occur (fig. 1). The process of seed infection at high humidity could be interrupted by low humidity and seed infection could still take place. The longer the interruptions were, however, the longer was the period of high humidity needed to obtain significant seed infection. Continual interruptions with low humidity essentially stopped seed infection. The effect of temperature on seed infection was examined in three groups of field grown pods with different amounts of natural *Phomopsis* infection. These had been stored at 50 percent relative humidity at 10°C for 3 months. They then were subjected to different numbers of days at 100 percent relative humidity in incubators set at 15, 20, or 25°C. After the appropriate high humidity periods, pods were transferred to 40 to 60 percent humidity within the same incubator. Seed infection was measured for all treatments 7 days after the high humidity period was started. The results (fig. 2) clearly showed that the rate of movement of *Phomopsis* from pod to seed increased with temperature. Increased seed infection was also associated with greater amounts of pod infection.

The combined effects of temperature, relative humidity, and podborne inoculum were examined in the field. High humidity was maintained in plots by sprinkler irrigation for 8 hours during daylight. Different plots in both early- and late-planted blocks of 'Amsoy 71' soybeans were so treated for 5 consecutive days in

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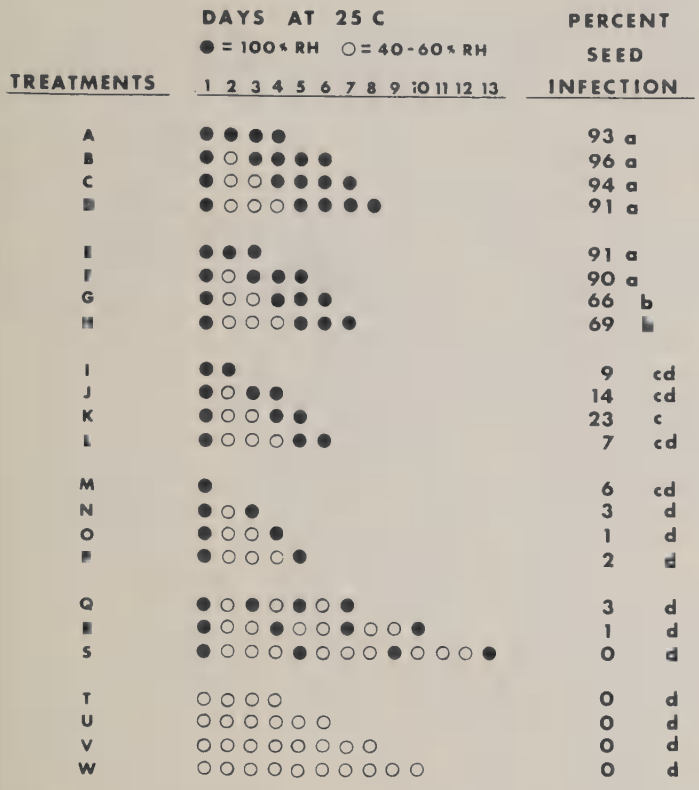

Figure 1.--Effects of different periods of high and low relative humidity (RH) on soybean pods artificially inoculated with Phomopsis sp.

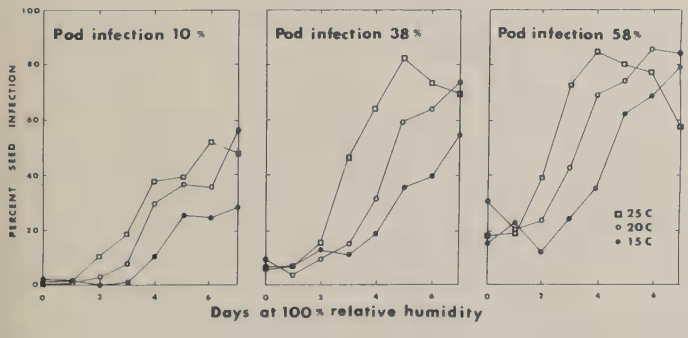

Figure 2.--Effect of temperature and number of days at 100 percent relative humidity on Phomopsis seed infection in soybean pods with different amounts of podborne Phomopsis inoculum.

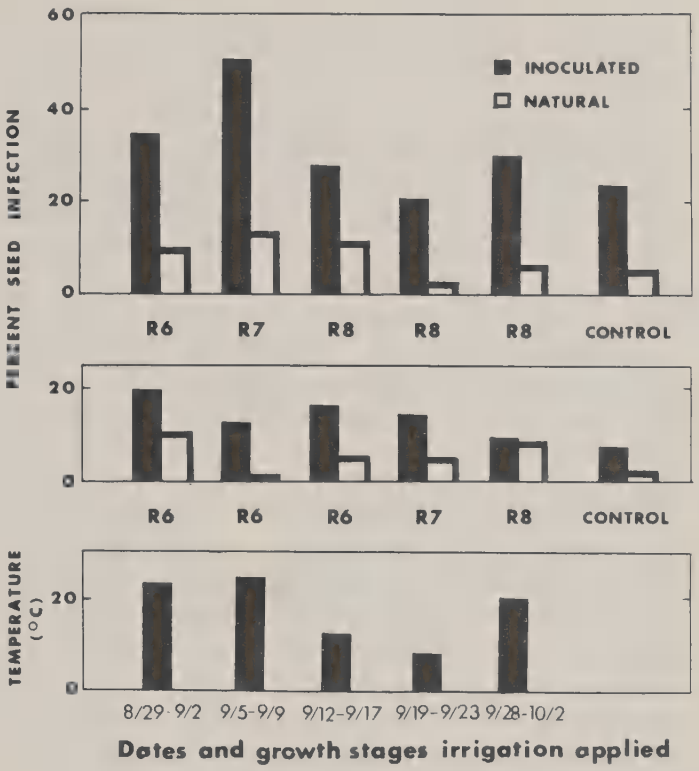

Figure 3.--Relationships between Phomopsis seed infection at harvest maturity and average daily temperature during irrigation periods in Phomopsis-inoculated and noninoculated field plots.

each of 5 weeks beginning in late August. Each plot consisted of noninoculated pods and pods inoculated at R5 with a spore suspension of Phomopsis. Seed infection was measured in inoculated and noninoculated subplots of all plots at harvest maturity. Temperature was measured throughout the course of the experiment. The results (fig. 3) for the early planting showed that high levels of seed infection occurred only when average daily temperatures during the period of irrigation were greater than 20°C. This effect was not seen in the second planting because the high temperature periods occurred at the R6 growth stage, when pods are not susceptible to infection, regardless of environmental conditions. Seed infection, in general, was higher in

inoculated than in noninoculated treatments.

This study clearly showed that Phomopsis seed infection is influenced by podborne inoculum and by temperature and relative humidity between R7 and harvest maturity. Periods of high relative humidity are essential for infection to occur, but higher temperatures can shorten the length of the period. Weather conditions favorable for seed infection, therefore, are more likely to occur in southern production areas and in plants maturing early in the growing season. This is in agreement with the pattern of disease severity normally observed. The data in this study suggest that the probability of average daily temperatures of 20°C and above occurring during the period when soybeans are maturing is a key factor in establishing critical pod infection levels for a particular geographical region.

Use of Podborne Inoculum to Predict *Phomopsis* Seed Decay

D.C. McGee[1]

Phomopsis seed decay can be controlled by application of fungicides to the growing seed crop. In the Northern United States, however, the disease often is not severe enough to justify treatment. A method to predict disease severity and then identify fields that should be sprayed would be of great value. Epidemiological data indicate that pods are a pathway for infection of seeds. Pod infection precedes seed infection, which does not occur to a significant extent until plants reach physiological maturity, and then only if prolonged periods of wet weather exist. These findings suggested that measurements of podborne inoculum might be used to predict severity of seed infection at harvest maturity. To determine whether this was feasible, however, more information was required on the epidemiology of the disease.

Because fungicides are ineffective after seeds are infected, the method requires that pod inoculum be measured, and the fungicide applied, before seed infection occurs. To determine the growth stage when this could happen, seed infection was induced in soybean pods at weekly intervals between growth stages R6 and R8. Pods, either naturally or artificially inoculated with *Phomopsis* sp., were detached from plants in the field, then held at 100 percent relative humidity and 25°C for 7 days. Seeds were then removed and plated on potato dextrose agar (PDA). Results (fig. 1) showed that extensive seed infection did not occur before R7. It also was necessary to know that the predictive measurement would not be invalidated by inoculum that subsequently reached pods. Artificial inoculation of pods with a *Phomopsis* isolate in the field and under growth chamber conditions showed that pods were susceptible to infection at R3 and R5 but not at R8 (table 1).

A quick, uncomplicated method was developed to measure *Phomopsis* inoculum on pods. This requires detaching pods at R6 and treating them sequentially in sodium hypochlorite and then a herbicide. They then are incubated at 100 percent relative humidity for 7 days at room temperature under constant light. Infected pods are identified by the presence of *Phomopsis* pycnidia. The equipment and materials for this test can be purchased from a hardware store. Within minimal training, seed company technicians can carry out the test themselves. Cooperative experiments with Iowa seed companies from 1981 to 1983 have demonstrated the practicality of the method and validated it in commercial soybean fields. Several Midwest seed companies have incorporated the method into their soybean seed production practices.

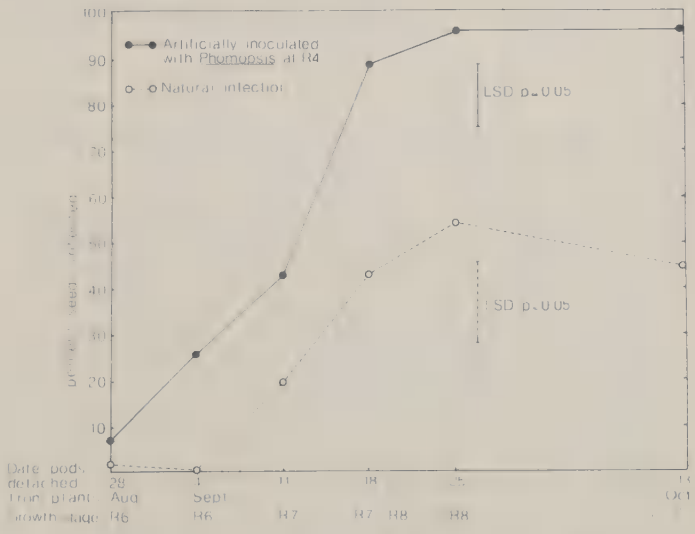

Figure 1.--Induced seed infection in relation to stage of growth of detached soybean pods, naturally and artificially inoculated with *Phomopsis*.

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Table 1 - Susceptibility of soybean pods and seeds to infection by *Phomopsis* sp. after inoculation of pods at different stages of growth.

Test Location	Growth stage when inoculated	Percent of pods infected 2 weeks after inoculation	Percent of seeds infected at harvest maturity	
			Plated immediately	Induced[a]
Field[b]	R2	56	8.5	90.6
	R4	93	28.5	95.9
	R5	80	22.4	96.9
	R8	27	.8	31.6
	Uninoculated	34[c]	4.0	42.3
	LSD (P=0.05)	10.7	19.0	12.2
Growth chamber[d]	R3	50	22.5	63.3
	R5	25	20.5	83.3
	R7	20	.0	35.0
	R8	0	.0	5.0
	Uninoculated	0	.0	.0
	LSD (P=0.05)	16.7	18.9	37.9

[a]Pods detached and maintained at 98 percent RH and 25°C for 1 week before seeds plated.
[b]All treatments planted on the same day; pods inoculated with a spore suspension of *Phomopsis* when appropriate growth stage was reached.
[c]Measured at R8.
[d]Planting dates staggered to allow inoculation of all treatments on the same day. Growth chambers maintained at 22 to 28°C and 70 percent RH.

Phomopsis Infection of Soybean Pods and Seeds as Influenced by Pod Nutrient Content

P. R. Thomison, D. L. Jeffers, and A. F. Schmitthenner[1]

Phomopsis seed decay is a disease associated with soybean senescence and maturation. Although symptomless *Phomopsis* infections occur in young pods early in the growing season, these infections remain dormant in the pod wall and have no adverse effect on seed until plants near maturity. Little is known concerning the physiological changes which occur in soybean plants during maturation which render them more susceptible to *Phomopsis* seed infection. The loss of natural resistance which immature pods appear to exhibit may be related to changes in the nutrient status of plant tissues which coincide with plant maturation. There is evidence in a number of host-pathogen interactions that nutrient deficiencies can predispose plants to increased disease. Potassium deficiencies in soybeans may increase the severity of *Phomopsis* seed infection, resulting in reduced germination. Greater *Phomopsis* infection of soybean seed in the lower halves of plants (vs. the upper halves) has led to speculation that such variation may be related to differences in the nutrient composition of pods from the upper and lower nodes. Although there is evidence that the nutrient composition of soybean plants changes during maturation, the effect of varying levels of pod nutrients on fungal seed infection has not been examined.

The principal objective of this investigation was to determine whether differences in the carbohydrate and mineral nutrient composition of pod tissue influence *Phomopsis* seed infection and its relationship to seed quality. Soybeans with different fruit loads were grown to generate varying levels of nutrients. It is well documented that reducing the sink size (that is, reducing pod numbers) of soybean plants results in a greater concentration of certain nutrients, including N, P, and total nonstructural carbohydrates, in the vegetative tissue. In this study, different pod removal treatments were used to obtain varying sink sizes. Certain stress conditions, such as K deficiencies and virus diseases, which increase *Phomopsis* infection, have also been reported to inhibit pod formation and to increase pod abortion and shedding.

Field experiments were performed at Wooster, OH over a 3-year period. Pod removal began when pods were less than 1 cm long and was repeated every other week until pod formation ceased. Plants were thinned to one plant per 30 cm of row to optimize growth and development. Side branches were removed from depodded plants to maximize fruit production on the main stem and to increase uniformity among plants. Treatments were as follows: one pod at every node; three pods at every node; no pods removed, side branches removed; and no side branches or pods removed. Cultivars representative of different maturity groups were grown to determine the interaction of maturation and pod removal on *Phomopsis* infection.

When plants reached harvest maturity, they were divided into their bottom, middle, and top thirds. Pods from each third were separated into seed and pod walls for seed

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quality, dry matter, and nutrient analysis. Seedborne fungi were identified on acidified potato dextrose agar (APDA).

The pod removal treatments had distinct effects on plant appearance and maturation. Stems and leaves of plants with reduced fruit loads remained green longer than those with no pods removed. Pod maturation and seed dry-down were delayed as much as 2 to 3 weeks in the one pod per node treatment.

In 1980, there were no marked differences in the incidence of Phomopsis seed infection among plants with varying pod numbers. However, moldy seed levels were significantly greater in treatments with reduced fruit loads. Moldy seed ranged from 17 to 61 percent in the upper and lower thirds of plants with one pod per node. With increasing pod numbers, the percentages of moldy seed dropped. In plants with no pods removed, moldy seed levels averaged less than 2 percent.

In 1981-82, reducing pod numbers resulted in significant differences in the incidence and severity of Phomopsis seed infection for both the early and late cultivars. Plants with pods removed consistently showed lower levels of germination and higher levels of Phomopsis infection and moldy seed than seed from treatments with no pods removed. For 'Wells', an early maturing group II cultivar, germination of normal seedlings in 1981 ranged from 12 percent, in the bottom third, to 49 percent, in the top third, in the one pod per node treatment; whereas seed germination was greater in the control plants (with no pods or branches removed), ranging from 65 percent in the bottom third to 97 percent in the top third. In 'Wells', Phomopsis seed infection ranged from 90 to 55 percent in the one pod per node treatment, in contrast to levels of seed infection ranging from 41 to 1 percent in the control. Differences in moldy seed levels among treatments were most evident in the bottom thirds of plants; in the one pod per node treatment, 46 percent of the seed were moldy, in contrast to 19 percent moldy in three pods per node treatments and 5 percent in the control. Plants with less altered fruit loads exhibited levels of Phomopsis infection, germination, and moldy seed that were intermediate between those of the one pod per node treatment and the control. Pod position also exerted a strong influence on the severity of Phomopsis infection in all treatments. Seed germination levels were lowest in the bottom third of plants where seed infection was greatest.

In 1981-82, the extent of Phomopsis pod infection at different growth stages (the green bean stage, R5; physiological maturity, R7; and harvest maturity, R8) was determined using a moist chamber incubation procedure (MCI). Pods were surface sterilized and dipped in paraquat to kill soybean tissue rapidly and stimulate pycnidia production. The percentage of pods showing Phomopsis pycnidia was recorded after 1 and 2 weeks of incubation. The results indicated greater Phomopsis infection of pods in the lower nodes at each developmental stage. There were also differences among pod removal treatments as to the percentage of pods with Phomopsis pycnidia present, with control plants generally exhibiting a greater percentage of pods with Phomopsis pycnidia. However, the MCI tests revealed much greater mycelial growth on pods from plants with reduced fruit loads, especially the one pod per node treatment. Mycelial growth was also more pronounced on pods from the lower nodes. The fungus responsible for the mycelial growth was not identified, although specimens of mycelium from pods plated on APDA yielded Phomopsis, Diaporthe, spp., and Fusarium spp.

Dry matter and nutrient accumulation in pod walls and seed was influenced by the pod removal treatments. Reducing fruit loads resulted in larger, heavier seed and pod walls. The larger seed of plants with reduced fruit loads was associated with seedcoat fissuring, that is, hypodermal cracks. The concentration of total non-structural carbohydrates (TNC), N, and P was significantly greater in pod walls from plants with reduced pod numbers. Pod removal had little effect on K, Ca, and Mg levels in pod walls. However, there were significant differences in levels of these nutrients at varying pod positions. K concentrations were highest in pod walls in the bottom third, whereas Ca levels were greatest in the top third. The depodding treatments had only slight effects on the accumulation of P, K, Ca, and Mg in seed. Total N in seed, averaged over different pod positions, was significantly greater in seed from the one pod per node treatments, whereas TNC values were lower in seed from plants with reduced fruit loads.

This study indicated that the greater Phomopsis infection and reduced germination of seed, which resulted from reducing pod numbers per plant, were associated with (1) slower plant maturation and seed dry-down; (2) higher levels of TNC, N, and P in pod walls; and (3) larger, heavier seed and pod walls. Seedcoat fissuring, another result of reducing fruit load, may have also been a factor which increased seed susceptibility to Phomopsis. Other studies suggest that seedcoat fissures may provide entry points for fungal invasion or enhance electrolyte leakage during seed germination, which stimulates greater fungal growth. The heavier pod walls and seed associated with pod removal treatments may have contributed to slower seed dry-down, providing the moisture-dependent Phomopsis more time to invade seed coats. Higher levels of TNC, N, and P present in senescing pod walls of plants with reduced pod number may have stimulated greater fungal growth in pods and subsequent infection of seed. Further study will be necessary to separate the effects of these various factors on Phomopsis in order to determine the contribution of each.

Effect of Moisture on Infection of Soybean Seeds by *Phomopsis* sp.

John C. Rupe and Richard S. Ferriss[1]

Pod and stem blight, caused by *Phomopsis* sp., is the most important disease affecting soybean seed quality in Kentucky. While infection of green plant tissues occurs throughout the growing season, seeds do not become infected until after physiological maturity, when the fungus grows from infected pods into the seed (Kmetz et al. 1978).

Seed infection in the field has been associated with warm, wet weather at the end of the season (Kmetz et al. 1979). In a growth chamber experiment, cool, moist conditions resulted in almost as much infection as did warm, moist conditions; but seed infection was very low when moisture was low irrespective of temperature (Spilker et al. 1981). TeKrony and co-workers (1983) found similar results in a field study. They developed a multiple regression model correlating seed infection with various weather variables. Manipulating the model, they determined that high relative humidity after physiological maturity had a much greater effect on seed infection than did temperature. Overall, these studies indicate that moisture after physiological maturity is a key environmental factor determining seed infection. However, the quantitative relationship between seed infection and moisture is unknown.

The purpose of this investigation was to determine the quantitative effect of moisture on seed infection. In order to do this, three objectives were addressed:

1. To determine the effect of pod moisture on the rate of seed infection.
2. To determine the effect of water potential on the growth of *Phomopsis* sp.
3. To establish the relationship between water potential and pod moisture so that the effects of water potential on seed infection rate and fungal growth rate could be compared.

Three plantings of cv. Williams soybeans were made at 3-week intervals in a field with heavy pod and stem blight disease pressure. These plantings provided pods of different maturities and moisture levels from which to choose at the end of the season. Pods at different moisture levels were obtained in two ways: either picked in the field after physiological maturity at different moisture levels (experiment 1) or picked at harvest maturity and rewetted to various moisture levels (experiment 2). At the time of picking, pod infection was high (82 to 100 percent) while seed infection was low (6 to 9 percent for the field collected pods and 10 to 37 percent for the rewetted pods). In either case, pods at particular moisture contents were incubated for various periods at 25°C, and then seeds were removed from the pods and were assayed on potato dextrose agar (PDA) amended with antibiotics and Tergitol NP10. Each moisture treatment was divided into 6 samples, each containing 30 to 35 pods. Data were taken as the proportion of seeds infected and were then transformed with the multiple infection transformation to give infections per seed (Van der Plank 1963).

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Table 1 - Percent moisture of 4 groups of pods collected in the field after physiological maturity (experiment 1) and of 5 groups of dry pods collected at harvest maturity and rewetted to various moisture levels (experiment 2).

Experiment 1		Experiment 2	
Mean	Standard deviation[a]	Mean	Standard deviation[a]
55	3	43	6
45	6	35	2
30	4	30	2
15	1	20	1
		17	1

[a]Standard deviation based on 3 replicates of 20 randomly selected pods each.

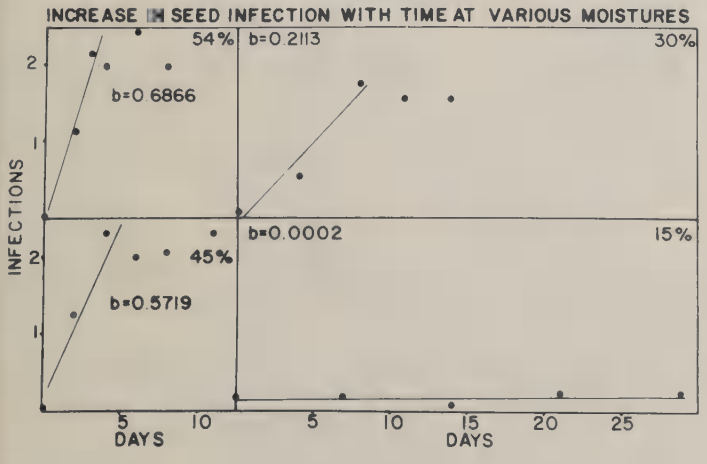

Figure 1.--Seed infection with time at pod moisture levels of 54, 45, 30, and 15 percent. The points represent the multiple infection transformation of the infection data and are the averages of 3 moisture-group replicates of 80 to 100 seeds each. Seed infection rates, in infections per day (b), were determined by fitting a straight line to those points showing increases in seed infection. Pods were obtained at the moisture levels indicated, directly from the field.

In experiment 1, pods were picked on the basis of pod appearance and feel to obtain groups with water contents of 55, 45, 30 and 15 percent (table 1). The results of one of three performances of this experiment are shown in figure 1. This figure shows the increase in seed infection with time: the Y axis is infections per seed and the X axis is the time for which the pods were incubated at the four moisture levels. Typically, there was a linear increase in infection with time until a plateau was reached, and then the number of seed infections varied along the plateau. The rate of seed infection was taken to be the slope of a line fitted to the points preceding the plateau. Seed infection rates calculated from all three replicates of experiment 1 are shown in figure 2. There was a great deal of variability among the replicates in the infection rates of the groups at 55 and 45 percent moisture, probably due to antagonism and competition by other micro-organisms. However, at 30 percent moisture, there was very close agreement in the seed infection rate among the three replicates. There was no increase in seed infection at 15 percent moisture. These results suggested that seed infection rate does change with moisture but that moisture levels from 20 to 40 percent needed further investigation.

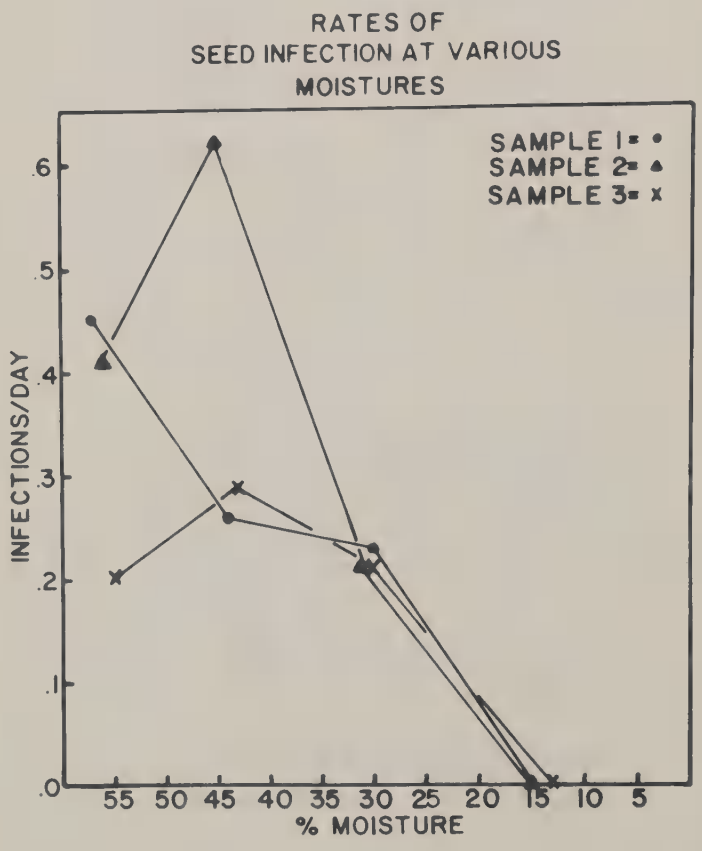

Figure 2.--Seed infection rate, in infections per day, plotted against percent pod moisture. Pods were obtained at various moisture levels directly from the field. Data are from 3 replicates of experiment 1.

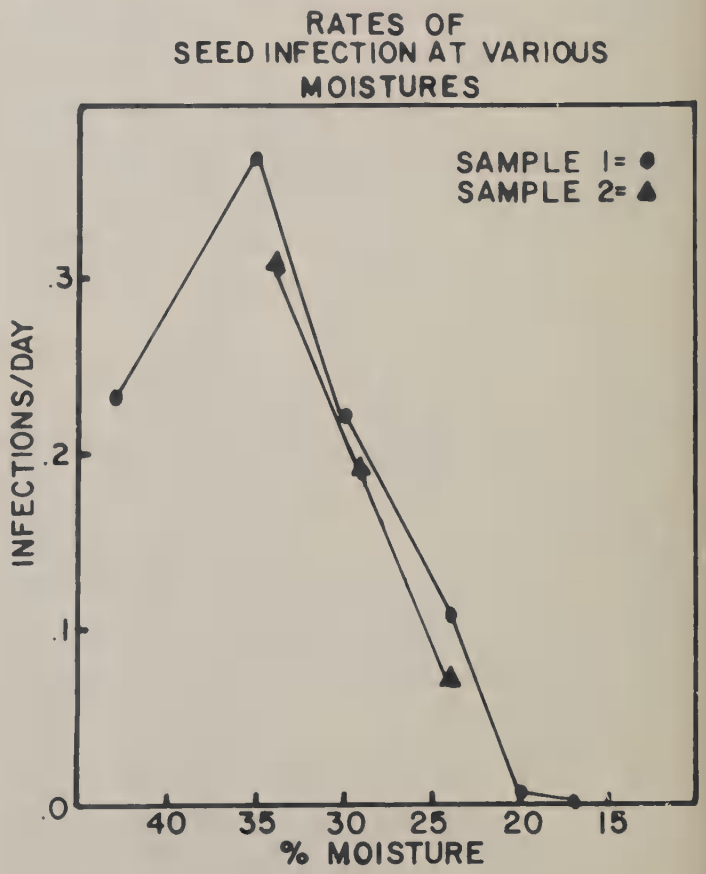

Figure 3.--Seed infection rate plotted against percent pod moisture. Data are from 2 replicates of experiment 2, in which pods were rewetted from 12 percent moisture to the moisture levels indicated.

To investigate seed infection from 20 to 40 percent moisture, dry pods (12 percent moisture) were collected and rewetted to moisture levels ranging from 17 to 43 percent (experiment 2, table 1). Seed infection progressed the same way in experiment 2 as in experiment 1. The seed infection rate showed a linear decline from 35 to 20 percent moisture, with an end point probably just below 20 percent (fig. 3). The rate of seed infection was lower at 43 percent than at 35 percent moisture, but 43 percent falls in the moisture range that, in experiment 1, had been associated with a great deal of variability in the seed infection rate. Combining the results of both experiments, an overall graph of the relationship of the seed infection rate to pod moisture level was obtained (fig. 4). There was very little change in the rate of seed infection from 55 to 35 percent moisture, but a linear decline in the seed infection rate from 35 to 10 percent moisture. These results indicated that Phomopsis sp. can infect seed under very dry conditions, as low as 20 percent moisture.

To determine the effect of water potential on the growth of Phomopsis sp., the osmotic potential of PDA was adjusted with either sucrose or KCl, and the radial growth of the fungus on the amended agar was measured. The fungus grew better on sucrose-amended PDA than on KCl-amended PDA, possibly due to a nutritive effect of the sucrose or a toxicity effect of the KCL (fig. 5). In either case, however, fungal growth rate increased rapidly when the osmotic potential was lowered from -3 to -10 bars, was optimum at from -10 to -30 or -40 bars, and then declined steadily to an end point somewhere between -180 to -190 bars. The permanent wilting point for most plants is approximately -15 bars.

To determine water potential of pods and seeds, the Shardakov (also known as the Chardakov) method was used. This method is based on changes in the density of sucrose solutions in which plant material has been immersed. By using a series of sucrose solutions representing a range of osmotic potentials, a range of water potentials containing the water potential of the plant material can be determined. Figure 6 shows the results of using this technique with seeds. The results were similar with pods, but somewhat more variable. An equation relating percent moisture to water potential was developed by relating the natural log of the negative water potential to percent moisture by linear regression. The equation for this line was used to express changes in seed infection with water potential instead of percent moisture, thus allowing a

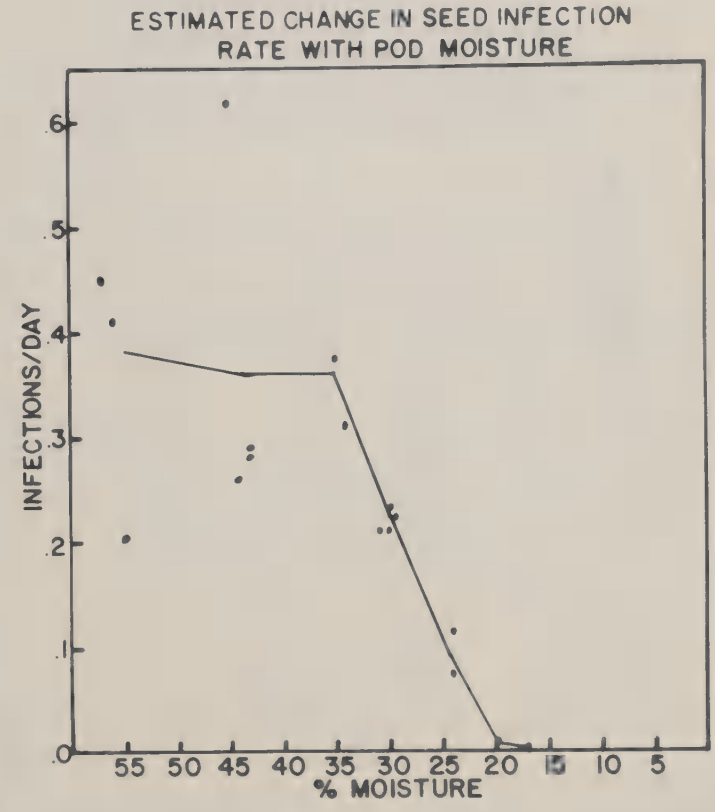

Figure 4.--Seed infection rate is plotted against percent moisture. The data are from experiments 1 and 2, involving either pods obtained from the field at particular moisture levels or picked at 12 percent moisture and rewetted.

comparison of seed infection rate with the fungal growth rate at various water potentials (fig. 7). The solid line represents changes in the fungal growth rate with osmotic potential on sucrose-amended PDA and the line with circles represents the seed infection rate at various water potentials. The optimum seed infection rate occurred at water potentials associated with optimal fungal growth rate and declined in a manner paralleling the fungal growth rate.

The close correlation which was observed between Phomopsis sp. growth in vitro and the seed infection rate may be taken to indicate that pod water potential is the primary factor governing seed infection after physiological maturity at moderate temperatures. Although other micro-organisms may affect the pathogen's activity at relatively high pod moisture contents (above 40 percent), the ability of Phomopsis sp. to grow at relatively low water potentials apparently allows it to largely escape their effects. If infection of green pods has occurred, then the amount of seed infection by Phomopsis sp. is probably determined by those weather variables (for example, relative humidity, rain, wind, and sunlight) which affect the rate of pod and seed drying after physiological maturity. Similarly, increases in seed infection with delayed harvest could be the result of pods and seeds being rewetted to moistures conducive for growth of the pathogen.

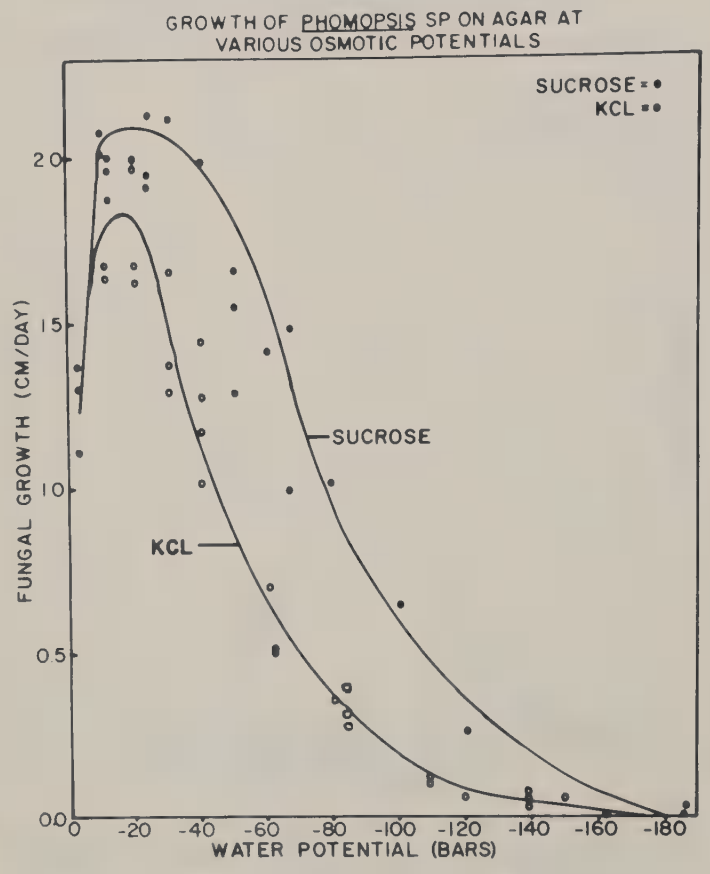

Figure 5.--Radial growth rate of Phomopsis sp. on potato dextrose agar (PDA) adjusted to various osmotic potentials with either sucrose (●) or KCl (○).

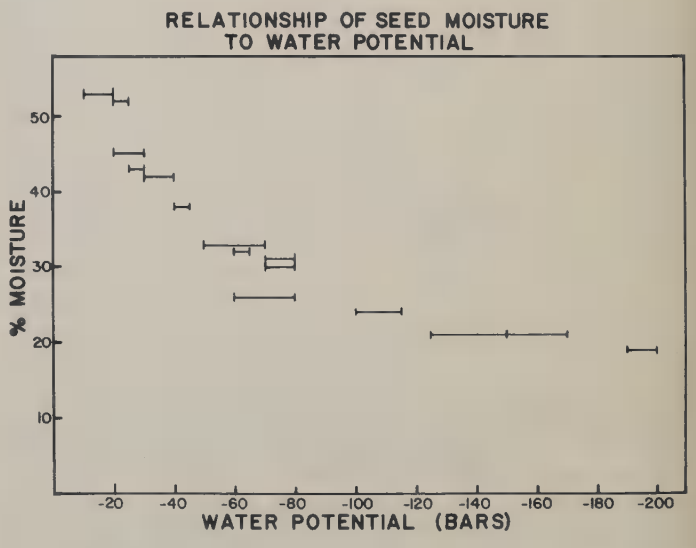

Figure 6.--Percent seed moisture at various water potentials. Water potential was determined using the Shardakov method (Barr 1969).

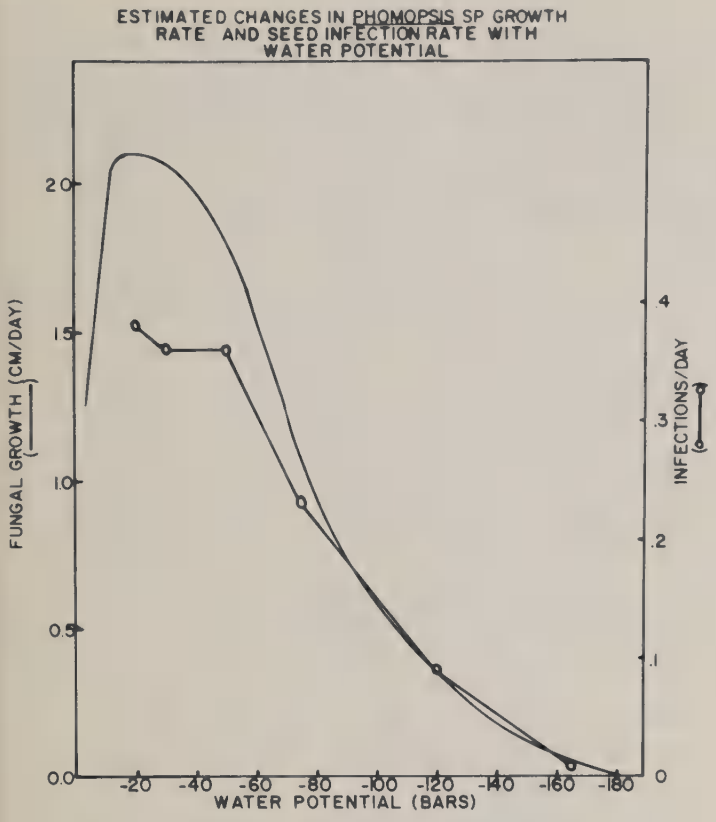

Figure 7.--Seed infection rate (0———0) and fungal growth rate (———) as a function of water potential. Fungal growth rate was measured on potato dextrose agar osmotically adjusted with sucrose.

References

Barr, H. D.
1969 Determination of water deficits in plant tissue. In T. T. Kozlowski, ed., Water deficits and plant growth, Vol. 1, p. 390. Academic Press, London.

Kmetz, K. T., C. W. Ellett, and
1979 A. F. Schmitthenner. Soybean seed decay: sources of inoculum and nature of infection. Phytopathology 69:798-801.

Kmetz, K. T., A. F. Schmitthenner, and
1978 C. W. Ellett. Soybean seed decay: prevalence of infection and symptom expression caused by Phomopsis sp., Diaporthe phaseolorum var. sojae, and D. phaseolorum var. caulivora. Phytopathology 68:836-840.

Spilker, D. A., A. F. C. Schmitthenner,
1981 and C. W. Ellett. Effects of humidity, temperature, fertility, and cultivar on reduction of soybean seed quality by Phomopsis sp. Phytopathology 71:1027-1029.

TeKrony, D. M., D. B. Egli, R. E. Stuckey,
1983 and J. Balles. Relationship between weather and soybean seed infection by Phomopsis sp. Phytopathology 73:914-918.

Pod and Seed Infection by *Phomopsis* Sp. During Soybean Seed Development and Maturation

L. J. Tomes, J. R. Hicks, and D. M. TeKrony[1]

Infection by *Phomopsis* sp. has been shown to be the primary cause of reduced seed quality in soybean seed. The primary objective of this study was to investigate the infection of soybean pods and seed by *Phomopsis* sp. at specific growth stages during seed development and maturation.

Field plantings of soybeans (cv. Williams) were made in mid-May of 1981, 1982, and 1983 in an area which had been in continuous soybean production for 3 or more years. This was done to insure that favorable environmental conditions and adequate inoculum for infection would be present. Pod and seed collections were made during soybean seed development and maturation at intervals based on pod and seed development (table 1). One pod containing 3 seeds was collected at approximately the 7th to 9th node from 70 plants at each growth stage. From each collection, 20 pods were used for moisture determination and 50 pods for fungal assay. Moisture determinations were made on a wet weight basis on both pods and seeds after drying at 105°C for 24 h. Pods for the fungal assay were rinsed in tap water to remove soil particles and surface contaminants and then were surface sterilized in 95 percent ethanol for 60 seconds and 0.5 percent NaOCl for 4 minutes. Pods were then rinsed in sterile distilled water and plated on acidified (pH 5.5) potato dextrose agar with streptomycin (100 p/m) added to inhibit

Table 1 - Pod color and seed characteristics of the various pod collections.

Growth stage	Pod color	Seed characteristics	Average time*
G	Green	Green--beginning seed	-27
G+	Green expanded	Green--fills locule	- 8
G/Y	Green/yellow	Green--yellow radicle	- 3
Y/G	Yellow/green	Green--yellow radicle	- 2
Y	Yellow	Yellow--55% moisture (PM)	0
EB	Early brown	Yellow--30% moisture	+ 3
LB	Late brown	Yellow--20% moisture	+ 8
HM	Harvest maturity	Yellow--14% moisture	+12
PHM	Postharvest maturity	HM + 2 weeks	+27

*Average number of days before (-) and after (+) physiological maturity (PM).

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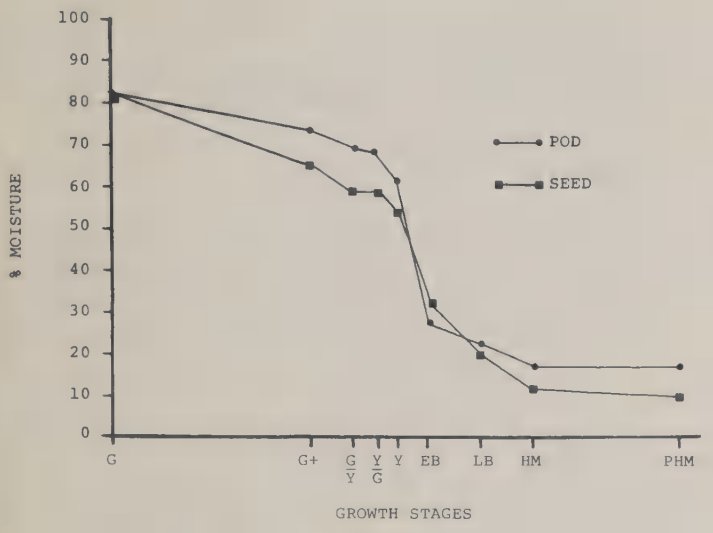

Figure 1.--Pod and seed moistures of the pod collections, 1981-83.

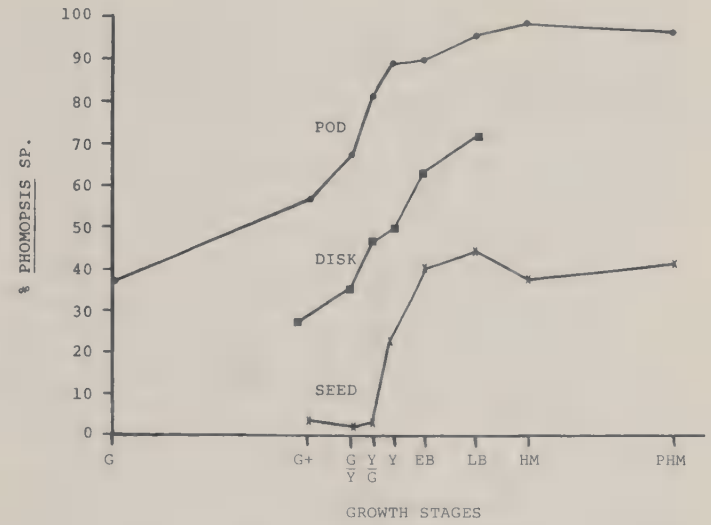

Figure 2.--Average Phomopsis sp. infection of pods, locular disks, and seed from control plants in 1981-83.

bacterial growth. The seed were aseptically removed and plated in order of their pod position (basal, middle, or apical). Each pod was then separated into its respective carpels, and a locular disk 1 cm in diameter was cut from the basal, middle, and apical locules of one carpel. The carpel and 3 locular disks were placed on one plate, maintaining the order of pod position of the disks. Evaluation of Phomopsis sp. infections was made after 7 to 10 days.

In 1982 and 1983, one half of the plants received an application of benomyl (1 lb A.I./acre) at the fully expanded green pod stage of plant development. After the fungicide application, collections for pod and seed moisture determinations and the fungal assay were taken at the same pod growth stages as the nontreated control. Similar trends in percent moisture and Phomopsis sp. infection were seen in each of the 3 years; therefore, only averages of the data for the 3 years will be presented.

Results

The first pod collection (G) was made in early August, at the beginning of seed development. Pod and seed moistures were each 82 percent (fig. 1), and Phomopsis sp. infection of the pods was approximately 40 percent (fig. 2) in the control sample. At the expanded green pod stage (G+), pod infection had increased to 65 percent. Average locular disk infection across all pod positions was 27 percent and seed infection was 3 percent. Pod infection increased with each collection to maximum levels of near 100 percent at harvest maturity, and increases were seen in disk infection. However, disk infection remained at levels below those found in the pods through the late brown (LB) stage. Seed infection remained at levels of less than 5 percent through the green (G), green/yellow (G/Y), and yellow/green (Y/G) pod stages. At the yellow pod (Y) stage, pod and seed moistures were 60 and 55 percent, respectively (fig. 1). This stage

represented physiological maturity (PM) of the seed and occurred in early September. As the pod began to senesce, there was a rapid decline in moisture, and pod moisture (27 percent) declined below seed moisture (32 percent) by the early brown (EB) pod stage. There was a sharp increase in seed infection as the pods lost green color and the seed reached PM (yellow color). Seed infection was 3 percent at the Y/G pod stage and increased to 40 percent by the early EB pod stage. This rapid increase in seed infection, which occurred as pods changed color from G/Y to brown, took place during a 5-day period centering around PM of the seed. Harvest maturity (HM), the first time seed moisture declined to 14 percent, occurred approximately 12 days past PM during the latter portion of September. At this stage, seed infection was 38 percent.

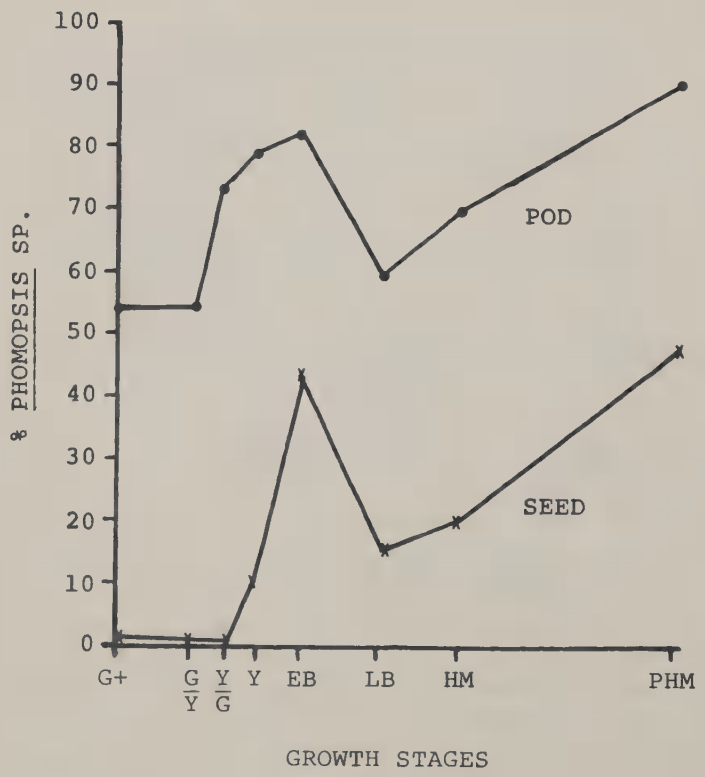

Figure 3.--Average Phomopsis sp. infection of pods and seed from benomyl treated plants in 1982-83.

Although seed were allowed to weather for 2 weeks past harvest maturity (PHM), infection did not increase above the levels found at the EB collection (40 percent).

Pod and seed infection in the benomyl treated plants followed similar trends as in the control from the G+ through the EB pod stages (fig. 3). However, following the EB stage, there was a sharp decline in both pod and seed infection in the benomyl treated samples. At the late brown (LB) stage, average pod and seed infection was 60 and 15 percent, respectively, which was approximately 20 percentage points below the infection levels in the control plants. From the LB stage on, Phomopsis sp. infection gradually increased, although infection levels at HM were still 20 percentage points less than in the nontreated control sample. The PHM sample had Phomopsis infection levels similar to those in the control, (that is, 90 and 40 percent Phomopsis infection in pods and seed, respectively). Seed quality improved following benomyl treatment by reducing Phomopsis infection; however, the

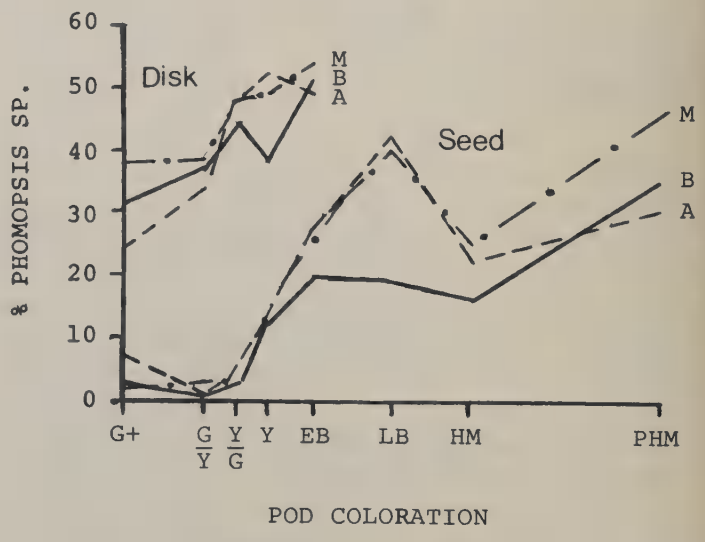

Figure 4.--Average Phomopsis sp. infection of locular disks and seed from the basal, middle and apical pod positions for 1982-83.

beneficial effect of the fungicide was negated by field weathering which occurred due to delayed harvesting.

Examination of locular disk and seed infection at the basal, middle, and apical positions of the pod indicated that Phomopsis sp. infection did not occur systemically (fig. 4). Similar percentages of Phomopsis infection were found in each of the three pod positions at each collection. There was a good relationship between infected locular disks and infected seeds in the same position. This would indicate that seed infection occurs due to penetration by fungal hyphae of the pod wall into the seedcoat rather than through any vascular connection.

Summary

This study showed that pod infection by Phomopsis sp. began early in seed development and reached levels >90 percent by physiological maturity (yellow pod). Increasing locular disk infection indicated that pods became more densely colonized by Phomopsis sp. as senescence occurred. Although pod infection was extremely high, little or no seed infection occurred during seed development (green to yellow pod color). However, favorable environmental conditions (high temperature, precipitation, or extended periods of high relative humidity) at the time pods lost green color and the seeds approached physiological maturity (55 percent seed moisture) resulted in rapid increases in seed infection. Application of the fungicide benomyl was effective in reducing both pod and seed infection by Phomopsis sp. by approximately 20 percentage points below untreated control samples at HM. A 2-week period of field weathering resulted in increased seed infection equal to that of the control. When locular disk and seed infection was examined based on respective pod positions, there was no preferential infection due to location. Likewise, Phomopsis sp. seed infection increased after the vascular connection between the plant and seed was severed by PM. This suggests that physical penetration by the fungus from the pod into the seedcoat was the primary means of seed infection.

SESSION C. EFFECTS ON SEED QUALITY

Peter R. Thomison, Chairman[1]

Phomopsis spp. Infection of Soybean Seeds Differing in Permeability

H. J. Hill, S. H. West, and K. Hinson[2]

Introduction

Phomopsis spp. (hereafter referred to as *Phomopsis*) are generally regarded as some of the most prevalent (3, 12) and pathogenic (8, 10) fungi associated with soybean [*Glycine max* (L.) Merr.] seeds. *Phomopsis*-infected soybean seeds have lower germination (8, 14) and lower seedling emergence (8, 10) than noninfected seeds. Often, the highest frequency of *Phomopsis* infection of soybean seeds occurs when the seeds remain in the field after harvest maturity (1, 15). Soybean seed infection by *Phomopsis* appears independent of seed-source inoculum (6) and mother-plant infection (1). Seed infection appears to be dependent on cropping history (6) and weather conditions (14). Seed infection under a postmaturation field weathering environment is believed to occur via infected pods from external sources (1).

Currently, there are fungicides that effectively reduce the probability of seed infection by *Phomopsis* and other pathogenic fungi (4, 5, 13), even when applied in the postmaturation or delayed-harvest field situation (5).

Fungicides, however, can be expensive to purchase and to apply. The best way to eliminate soybean seed infection by *Phomopsis*, along with other pathogens, is to identify sources of resistance and to breed resistance into modern cultivars. One such type of resistance mechanism could be a mechanical barrier to infection. Since soybean seed infection can be higher after plant senescence, a mechanical barrier mechanism will continue to be effective, whereas metabolic-based resistance may lose effectiveness due to reduced biochemical activity after senescence.

Since penetration by fungi is thought to occur first in the soybean pod, a thick pod wall or a pod wall with an impenetrable barrier may prevent subsequent seed infection in the field. But pods are discarded, and only seeds are stored; so an impenetrable barrier may be more effective if located in the seedcoat rather than the pod.

Our approach to the soybean seed quality problem is to identify characteristics that will result in improved resistance to seed deterioration. Because *Phomopsis* infection is often associated with field deterioration, any improved resistance to deterioration must also include *Phomopsis* resistance. Among the characteristics that may improve resistance to deterioration in soybean seeds is the impermeable seedcoat. Previous reports have demonstrated the ability of the impermeable seedcoat to prevent both field (11) and storage (9) deterioration in soybeans. Although impermeable seeds or "hardseeds" are found in seed lots of modern cultivars, the impermeable-seedcoat trait genetically induces the impermeable condition in seeds to much higher frequencies than occur naturally in seed lots. In these genetic lines, more seeds in a lot will be impermeable. This report will propose a way that the impermeable

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seedcoat may prevent Phomopsis infection of soybean seeds, followed by discussion of the results of an experiment that shows the ability of impermeable seeds to prevent Phomopsis infection and retain viability in the field.

Scanning Electron Microspcopy Studies

Using scanning electron microscopy (SEM), selected features of the soybean seedcoat of modern cultivars have been characterized. Some studies have shown that the seedcoat surface is covered with indentations that we refer to as "pores" (2, 16, 17). These surface pores occur on seeds of different cultivars and vary in frequency. The highest frequency reported has been for 'Hardee' seeds [27.7 pores per 0.075 mm^2 (2)].

SEM studies with 'Hardee' seeds have revealed that pores are abundant over the entire surface of the seedcoat except in areas immediately adjacent to the hilum. Pores varied in size and shape; the dimensions of the elongated pores can be 3 by 40 μm. The larger round pores can be 7 μm in diameter. Often, pores will terminate in slotlike openings deep within the palisade layer of the seedcoat. Some investigators have associated cavities within the palisade layer with surface pores (17, 18). Fungi have been thought to enter the soybean seedcoat through cracks or large holes caused by weathering or insects. We hypothesize that these naturally occurring surface pores also may provide an entry into the seedcoat. In fact, we have observed hyphae entering the seedcoat through these pores (7). Hence, these naturally occurring pores observed on seedcoats of modern cultivars may provide a pathway for infection independent of visible seedcoat defects.

By comparing the seedcoat surface of a cultivar with the surface of a known impermeable seed, we have observed two important differences. First there are a greatly reduced number of pores on the surface of an impermeable seedcoat. Second, the surface of an impermeable seed has wax-like globules and wax-like material embedded in it. Whether these wax-like formations on the surface of impermeable seeds are the cause of the impermeable condition is unknown. The important point is that the surface morphology of an impermeable seed appears to offer a better mechanical barrier to fungal penetration than does the surface of a normal seed.

Field Studies

The SEM observations were followed by a field trial to determine the effectiveness of seeds having the impermeable seedcoat to resist Phomopsis infection. Two advanced-F_4, impermeable-seed breeding lines (8731 and 8745) were selected, with 8745 maturing 2 weeks earlier than 8731. Cultivar Hardee was used as a control. Seeds of these lines were first harvested at harvest maturity and then 2 months later. The 2 months of delayed harvest was imposed to insure that these seeds were exposed to an adequate disease level and environmental stresses.

At harvest maturity line 8745 seeds were lower in germination and higher in frequency of Phomopsis infection than line 8731 seeds. The quality of line 8745 seeds was probably lower because they were exposed to more rainfall prior to maturity. The delayed harvest of 2 months significantly reduced germination and

significantly increased the frequency of Phomopsis-infected seeds for both lines. However, germination of line 8731 seeds, which were exposed to the same rainfall as 'Hardee', was three times higher.

To determine the effectiveness of the impermeable seedcoat in resisting Phomopsis infection, line 8731 and line 8745 seeds from both harvests were separated into classes based on their permeability (since not all line 8731 and 8745 seeds were impermeable). Frequency of Phomopsis infection and germination were determined for seeds within each group. To separate seeds within permeability classes, seeds were soaked in water for periods of 2 and 24 hours. If seeds were observed to imbibe water after 2 hours of soaking, they were classified as "very permeable." If seeds were observed not to imbibe water after 2 hours of soaking, they were classified as "slightly permeable." If seeds were observed not to imbibe water after 24 hours of soaking, they were classified as "impermeable." At maturity, germination and frequency of Phomopsis infection did not differ for seeds among the permeability classes for either line. Germination and frequency of Phomopsis infection were similar to impermeable line means. Two months of delayed harvest, however, caused differences in germination and frequency of Phomopsis infection among these seed permeability classes (that is, across lines). The very permeable seeds of lines 8731 and 8745 had a significantly higher frequency of Phomopsis infection and were significantly lower in germination than either the slightly permeable or impermeable seeds. The slightly permeable seeds had a significantly higher frequency of Phomopsis infection and were significantly lower in germination than the impermeable seeds.

During the 2 months of field weathering, very permeable seeds (across lines) decreased from 80 to 12 percent germination and the frequency of Phomopsis infection increased from 13 to 51 percent. Slightly permeable seeds decreased in germination from 83 to 66 percent germination and the frequency of Phomopsis infection increased only from 13 to 17 percent. Impermeable seeds were not affected by the delayed-harvest stress. Germination of these seeds actually increased from 78 to 83 percent and the frequency of Phomopsis infection decreased from 14 to 11 percent.

In conclusion, any expression of the impermeable-seedcoat trait imparted some resistance to Phomopsis infection and increased viability. The resistance to Phomopsis infection and retention of viability persisted in impermeable seeds under the stress of delayed harvest. These results confirmed the hypothesis that the seedcoat surface of the impermeable seed may impart resistance to fungal penetration.

Seeds that carried the impermeable-seedcoat trait but were permeable deteriorated. The seedcoat surface morphology of very permeable or slightly permeable seeds is unknown, and further research is needed to compare their surface with the impermeable seed surface.

These studies support the concept that better protection of the soybean seed embryo may come from an improved seedcoat character.

References

1. Athow, K. L., and F. A. Laviolette. 1973. Pod protection effects on soybean seed germination and infection with Diaporthe phaseolorum var. sojae and other microorganisms. Phytopathology 63:1021-1023.

2. Calero, E., S. H. West, and K. Hinson. 1981. Water absorption of soybean seeds and associated causal factors. Crop Sci. 21:926-933.

3. Dunleavy, J. M. 1976. Pathological factors affecting seed germination. In L. D. Hill, editor, World soybean research, p. 426-470. Interstate Pub., Danville, IL.

4. Ellis, M. A., M. G. Ilyas, F. D. Tenne, and others. 1974. Effect of foliar applications of benomyl on internally seedborne fungi and pod and stem blight in soybean. Plant Dis. Rptr. 58:760-763.

5. Ellis, M. A., and J. B. Sinclair. 1976. Effect of benomyl field sprays on internally borne fungi, germination, and emergence of late harvested soybean seeds. Phytopathology 66:680-682.

6. Garzonio, D. M., and D. C. McGee. 1983. Comparison of seeds and crop residues as sources of inoculum for pod and stem blight of soybeans. Plant Dis. 67:1374-1376.

7. Hill, H. J., and S. H. West. 1982. Fungal penetration of soybean seed through pores. Crop Sci. 22:602-605.

8. McGee, D. C., C. L. Brandt, and J. S. Burris. 1980. Seed microflora of soybeans relative to fungal interaction, seedling emergence, and carry over of pathogens to subsequent crops. Phytopathology 70:615-617

9. Minor, H. C., and E. H. Paschal. 1982. Variation in storability of soybeans under simulated tropical conditions. Seed Sci. Tech. 10:131-139.

10. Paschal, E. H., and M. A. Ellis. 1978. Variation in seed quality characteristics of tropically grown soybeans. Crop Sci. 18:837-840.

11. Potts, H. C., J. Duangpatra, W. G. Hairston, and J. D. Delouche. 1978. Some influences of hardseededness on soybean seed quality. Crop Sci. 18:221-224.

12. Sinclair, J. B.
1975 Some important seed- and soil-borne bacterial and fungal pathogens of soybeans. In D. K. Whigham, editor, Soybean production, protection, and utilization, p. 124-131. Proc. of Conf. for Scientists of Africa, the Middle East, and South Asia. INTSOY Series No. 6, Univ. of Illinois, Urbana.

13. Sinclair, J. B.
1976 Seed-borne bacteria and fungi in soybeans and their control. In L. D. Hill (editor), World soybean research, p. 470-478. Interstate Pub., Danville, IL.

14. Wallen, V. R., and W. L. Seaman.
1963 Seed infection of soybean by Diaporthe phaseolorum and its influence on host development. Can. J. Bot. 41:13-21.

15. Wilcox, J. R., F. A. Laviolette,
1974 and K. L. Athow. Deterioration of soybean seed quality associated with delayed harvest. Plant Dis. Rptr. 58:130-133.

16. Wolf, W. J., and F. L. Baker
1980 Scanning electron microscopy of soybeans and soybean protein products. Scanning Electron Microscopy 3:621-634.

17. Wolf, W. J., F. L. Baker, and
1981 R. L. Bernard. Soybean seedcoat structural features: pits, deposits, and cracks. Scanning Electron Microscopy 4:531-544.

18. Yaklich, R. W.
1983 Moisture movement into impermeable soybean testa. Agron. Abstr. p. 122, Madison, WI.

Heritability of Tolerance to Diaporthe/Phomopsis Seed Mold of Soybean

T. R. Anderson and R. I. Buzzell[1]

Species of Diaporthe and/or Phomopsis are the most prevalent and economically important seed decay fungi of soybean in Ontario, Canada. Recent surveys (unpublished) of seed lots from seed production fields indicate an average incidence of 17 percent seed infection (range 0 to 61 percent).

Current measures to control seed mold in Canada are limited to choosing full-season cultivars, crop rotation, and timely harvest. Although resistance or tolerance to seed mold has not been characterized genetically, differences in the incidence of infected seed among cultivars have been noted in the literature, which suggests that variation in tolerance exists among soybean genotypes. Three factors known to increase the incidence of seed mold are the earliness in maturity of a cultivar in relation to the length of the growing season, delayed harvest, and warm moist weather during pod fill and dry-down. The effect of genotype on the incidence of seed mold has not been determined.

In the past, attempts to screen for tolerance to seed mold have been based on a visual inspection of breeding material and assignment of an arbitrary value for seed quality or appearance. Few research projects involving specific screening of soybean germplasm for tolerance have been based on actual infection as determined from incubation in moist towels or on an agar medium. A screening program involving 20 lines (1 from Minnesota and 19 from Harrow) and 10 cultivars was initiated at Harrow in 1980. The objective of this study was to determine the influence of genotype on the incidence of seed infection and to identify other factors which may influence the variability of infection.

The lines and cultivars ranged in maturity requirements from 90 to 126 days (1979 test results at Harrow) and originated from a genetically diverse background. The nursery was planted in hill plots, six plants per hill, with a hill spacing of 60 by 60 cm and four replicates. To determine the degree of seed infection, 25 seed per hill plot were surface sterilized, plated on potato dextrose agar, and incubated for 5 to 7 days at 22°C. Analysis of data was based on total infection by Diaporthe phaseolorum var. caulivora and D. phaseolorum var. sojae and/or Phomopsis sp. The correlation coefficient of percentage infection over 4 years with the 1979 data on days to maturity was highly significant (r=-0.52). Infection values were adjusted to a mean maturity on a replicate basis by linear regression techniques prior to analysis of variance.

In 1980 and 1981, harvest was delayed until December and November, respectively, to promote development of seed mold (1). Incidence of seed mold was higher in 1980 than in 1981 (table 1). Analysis of the data over the 2 years resulted in highly significant (P=0.01) year (F=106.0) and variety (F=9.0) differences with no significant Y x V interaction (F=1.5).

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Table 1 - Incidence of seed mold on 30 soybean cultivars/lines at Harrow, Ontario.

Cultivar	Maturity*	Percent seed infection**				$\bar{x}$
		1980	1981	1982	1983	
GNOME	126	10	2	16	5	8
OX618	119	7	1	32	9	12
OX314	108	8	3	29	16	14
OX611	100	24	6	9	19	15
OX615	122	10	6	29	14	15
M405	121	17	6	29	19	19
OX697	113	7	3	55	9	19
OX312	125	22	4	17	37	20
OX324	122	9	4	48	22	20
OX299	110	9	6	43	25	21
OX315	122	13	3	52	15	21
HARCOR	126	26	14	31	13	21
OX329	126	23	8	27	32	23
OX328L	124	22	17	28	27	24
EVANS	111	39	7	43	19	27
DAWN	124	38	8	48	15	27
OX708	114	19	9	68	14	28
MAPLE ARROW	104	27	18	62	7	29
OX692	125	37	26	34	19	29
OX307	116	26	12	53	24	29
HODGSON	117	21	18	57	20	29
PREMIER	124	31	7	60	19	29
HAROSOY 63	124	33	19	51	19	31
OX634	112	24	15	69	15	31
STARBUCK	119	41	15	53	21	33
OX610I	123	60	35	26	16	34
OX328E	108	29	21	75	19	36
OX619	100	59	24	35	38	39
OX311	122	42	23	77	21	41
COLES	122	36	23	78	29	42
$LSD_{0.05}$		16	12	20	15	8
MEAN		26	12	45	18	25
C.V.(%)		45	74	32	56	46

*Days from planting to maturity, 1979.
**Values were adjusted by regression to remove the effect of maturity differences among cultivars/lines.

Table 2 - Minimum, maximum, and average visual counts of infected seed and correlations between visual counts and actual infection determined by surface sterilization and plating on PDA.

	Visual (%)			Actual			
Year	Min.	$\bar{x}$	Max.	Min.	$\bar{x}$	Max.	r
1980	0	3	17	7	26	60	0.83**
1981	0	2	11	1	12	35	.71**
1982	1	6	15	9	45	78	.15
1983	1	4	6	5	18	38	.49**

**P = 0.01.

In 1982 and 1983, plots were irrigated in an attempt to increase the incidence (2) and uniformity of infection, thereby avoiding a delayed harvest. Corn was planted around the nursery to increase humidity, and the hill plots were subjected to overhead irrigation for 1 h each morning and evening from July 15 until harvest. Entries were divided into four groups and harvested approximately 2 weeks past maturity. This involved the period from September 10 to the last week of October.

In 1982, mean percentage of infected seed was higher and the C.V. lower than in 1980 or 1981, when plants were harvested late, and indicated an advantage for irrigation. However, in 1983, results were similar to 1981. Abnormally hot, dry weather in August may have contributed to low infection rates and increased variability. Irrigation was useful in increasing seed mold. Mean incidence of seed mold in nonirrigated plots of 10 cultivars in a separate experiment was 8 percent as compared to 21 percent in irrigated plots. Analysis of variance of the 1982-83 results indicated highly significant (P=0.01) year (F=2.39) differences and a highly significant Y x V interaction (F=5.5). There were no variety differences when tested by the Y x V mean square.

In the combined analysis of the 4-year results, there was a highly significant Y x V interaction and significant (P=0.05) differences among varieties when the Y x V interaction was used as an error term.

The broadsense heritability (a measure of the genotypic contribution to the results) was 82.8 percent (1980, 1981), 56.4 percent (1980, 1981, 1982) and 53.5 percent (1980, 1981, 1982, 1983).

Screening large numbers of lines would be more efficient if it could be accomplished visually, without resorting to actual counts as determined by plating and incubation. There was a moderate correlation between actual infection and visually determined infection (100 seed per hill plot) in 3 of 4 years (table 2), but visual ratings were always less than actual ratings and provided little range from which tolerant lines could

be selected and susceptible material discarded.

Three of the entries, OX314, OX618 and Gnome, have determinate (dt1 dt1) growth which may have had some effect on the incidence of seed mold.

TeKrony et al. (3) observed less seed mold in determinate OX303 than in indeterminate 'Beeson'. Three of the varieties were not affected by seedcoat mottling caused by soybean mosaic virus; these were OX314 (Im), OX615 (Rsv2) and OX312 (self-buff seedcoat), but there was no indication that seedcoat mottling affected the incidence of seed mold. For example, the correlation between seedcoat mottling and incidence of seed infected with mold for the other 27 entries in 1982 was not significant (r=0.19).

Our results suggest that a combination of irrigation and delayed harvest is needed in screening soybeans for improved tolerance to seed mold. Unless the genotype x environment interactions can be reduced, more than one environment (year) will be required. Also, selection would need to be made in relation to set maturity standards because of maturity effects. The possibility of using natural selection in conjunction with these techniques needs to be explored as a means of improving the seed mold tolerance of breeding populations.

Conclusions

1. Differences in susceptibility to Diaporthe/Phomopsis seed mold exist among soybean seed lines.

2. Broadsense heritabilities are sufficiently high to permit selection, but the variety x year interactions indicate a need for years and/or locations.

3. Further research is required on the suitability of late harvest and irrigation in selecting seed-mold-tolerant cultivars differing in maturity requirements.

4. The effect of maturity date on seed mold requires further definition. Evaluation of isolines differing in maturity requirements may prove beneficial.

References

1. Wilcox, J. R., F. A. Laviolette, 1974 and K. L. Athow. Deterioration of soybean seed quality associated with delayed harvest. Plant Dis. Reptr. 58:130-133.

2. Ross, J. P. 1975 Effect of overhead irrigation and benomyl sprays on late-season foliar diseases, seed infection, and yields of soybean. Plant Dis. Reptr. 59:809-813.

3. TeKrony, D. M., D. B. Egli, 1984 J. Balles, and others. Effect of date of harvest maturity on soybean seed quality and Phomopsis sp. seed infection. Crop Sci. 24:189-193.

Effect of *Diaporthe/Phomopsis* on the Reproducibility of Soybean Germination Tests

D. V. Phillips[1] and W. R. Guerke[2]

Soybean seed lots containing three different levels of internal *Diaporthe/Phomopsis* were sent for germination tests (rolled towel) to 30 seed laboratories as part of an Association of Official Seed Analysts (AOSA) referee test (REF). Fourteen of these laboratories also determined germination by an accelerated aging test (AA). At the same time, 19 of these laboratories were sent routine service samples (SER) from the same seed lots. The high quality, medium quality, and low quality seed had germination rates of 93, 80, and 66 percent, respectively, and internal *Diaporthe/Phomopsis* percentages of 3, 17, and 45, respectively. The percentage of other internally borne organisms was similar for the three seed lots. The coefficient of variability and the percentage difference between the high and low germination reports were at least twice as great with the low quality seed lots as they were with the high quality seed lot, for each of the three tests. The percentage of laboratories which reported significantly different germinations for the REF and SER tests was 15 percent high quality seed and 39 percent for the low quality seed. Using the AOSA tolerance test used for regulatory purposes, 11, 21, and 32 percent of the laboratories reported results of the SER test out of tolerance with the REF test for the high, medium, and low quality seed lots, respectively. Similar results were obtained comparing the REF to the AA test and the SER to the AA test. All comparisons indicated that variation in the germination tests results, both within and between laboratories, was much higher with seed lots containing high levels of *Diaporthe/Phomopsis*.

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Disease Symptoms in Germination Tests of Soybean

James F. Schoen[1]

According to the rules of the Association of Official Seed Analysts (AOSA), soybean seeds in rolled towel tests are incubated at a constant 25°C or at alternating temperatures of 30°C for 8 hours and 20°C for 16 hours. A preliminary count on day 5 is recommended, at which time most of the seedlings of a vigorous sample would be removed and counted. Water may be added, and another 3 to 5 days is allowed for development of smaller seedlings. A seedling is considered abnormal if it has a decayed epicotyl, provided the decay has spread from the cotyledons and is not due to improper test conditions. Slight infection is ignored if essential seedling parts have not been seriously damaged as to impair their function, especially the growing point. Vascular tissue could also be considered an essential part, and judgments may require dissection of the hypocotyl to note the extent of a decayed area.

Seeds severely infected with Phomopsis decay into a slimy mass with some woolly, white hyphae and some dark areas, which may be precursors of pycnidia or may indicate saprophytes such as Alternaria or Cladosporium. These seeds sometimes show symptoms of "moldy bean" before they are planted. If the disease is expected, these dead seeds should be removed from the test as early as possible, probably at about the fourth day. Seedlings may be retarded in their development by severe infection of the cotyledons at their point of attachment to the hypocotyl and at the growing point of the epicotyl. Hypocotyl lesions from moderately infected seeds may become severe, but sometimes the cotyledons escape infection. A combination of mechanical injury aggravated by a Phomopsis infection has also been observed. Mild infection of the hypocotyl and spots on cotyledons are symptoms which appear in towels but do not occur in sand, soil, or soil substitutes such as Redi-Earth. A range of decay may occur on both hypocotyls and cotyledons, but if the decay is not extensive and the epicotyls are not discolored, seedlings would be included in the germination count.

Fusarium is occasionally a serious problem on the Delmarva peninsula and is easily confused with Phomopsis in the towel test. Soft decay caused by Fusarium is somewhat similar to Phomopsis, but hyphae are usually more profuse than with Phomopsis. Various amounts of decay on cotyledons with healthy primary leaves protruding and epicotyls apparently noninfected are typical of a slight Phomopsis infection from the seedcoat. The descriptions in the AOSA rules do not address this type of seedling infection because they do not specify how much decay constitutes an abnormality.

In contrast, a few samples were found with a mild discoloration of the cotyledons but with primary leaves that were necrotic. Infection extended into the epicotyl--a critical growth region of the seedling. These seedlings would not be included in the normal count if it was determined that the decay had spread from the cotyledons. No Phomopsis or other micro-organisms could be isolated from

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the decayed areas by plating them on agar media. This decay stopped development of the epicotyl in towel tests, but the symptoms did not develop in soil tests. Symptoms increased with this seed lot when the towel tests were placed in closed plastic boxes to attain a high humidity. The cause in this particular seed lot appeared to be a physiological weakness that was expressed under certain test conditions.

In conclusion, when our laboratory discovers severely infected seedlings in rolled towels, we generally use a soil substitute such as Redi-Earth or Pro-Mix for comparative tests. The results of soil tests can be useful as guidance for the proper evaluation of seedlings in the towel test because soil gives a better indication of the pathogenicity of seedborne organisms. Also, we have shown that there are several causes of decay in soybean towel tests and that they cause difficulties in seedling interpretation. This is an area which requires better guidance in the AOSA rules.

Effect of *Phomopsis* spp. on Soybean Seed Quality in Brazil

Ademir Assis Henning and José de Barros França Neto[1]

Pod and stem blight occurs naturally in most soybean production areas in Brazil. The disease has not severely affected crop yield, but seed quality is frequently reduced especially when rainfall associated with high temperature occurs during seed maturation.

During the 1979-80 growing season, a severe outbreak of *Phomopsis* seed infection was observed in some regions of the country, mainly in the States of Parana, Sao Paulo, and Minas Gerais. Consequently, large numbers of seed lots were reported to have poor viability as determined by the standard germination test (rolled paper towels).

Seed samples of many cultivars from different localities were collected and tested at the National Center for Soybean Research (EMBRAPA-CNPS). Seeds were evaluated in the standard germination test (at constant temperatures of 25 and 30°C), blotter test, tetrazolium test, and soil emergence in the greenhouse. All these tests were performed comparing fungicide treated seed, and surface-sterilized (sodium hypochlorite) seed with nontreated seed. *Phomopsis* was found to be the cause of low germination in vitro, although in some samples the occurrence of *Fusarium* spp. (mainly *F. semitectum*) and *Cercospora kikuchii* was also observed.

1) EMBRAPA - National Center for Soybean Research. Caixa Postal, 1.061. 86.100 - Londrina, PR. Brazil.

In the laboratory, germination percentages of contaminated seed treated with fungicides were similar to seedling emergence percentages in greenhouse tests of nontreated seed samples in soil. These results may be explained as due to an escape mechanism by which seedlings leave infected seedcoats in the soil, while in the standard germination test they remain in close contact with the cotyledons which causes them to decay.

In those lots in which germination was not improved by the seed treatment, the tetrazolium test showed that the problem was due to one or more of the following factors: Mechanical damage, weathering, or stinkbug injury. These results showed that under conditions leading to *Phomopsis* spp. infection, the standard germination test alone becomes inadequate. The use of fungicide-treated seeds or the sand emergence test would be alternatives to consider in these cases. Otherwise, a seed checkup using the blotter and tetrazolium tests would be the best way to precisely determine the causes of the problem.

Using this recommendation, it was possible to save, during the 1980 planting season, around thirty thousand tons of seed in the State of Parana alone.

The fact that *Phomopsis* spp. have no effect upon field emergence under favorable soil conditions had already been noticed in 1979. Seed samples of cultivar IAC-7 with infection levels of 35.8 and 68.8 percent were treated with different fungicides and sown in the field. The best treatment (thiram 2.1 g a.i./kg) resulted in 95.5 and 97.0 percent emergence, whereas for the check it was 94.0 and 95.3 percent

for the high and low levels of seed infection, respectively.

To explain this, two hypotheses were considered: (1) the isolates (or species) of the fungus present in the seeds are not pathogenic and (2) favorable conditions for rapid germination and field emergence allow the seedling to escape from the infected seedcoat. With the purpose of testing these two possibilities, another experiment was conducted.

Several isolates differing in colony morphology were obtained from the same seed sample and used to artificially inoculate 'Davis' soybean seeds. There was much variation in pathogenicity among the isolates that affected seedling emergence differently in the greenhouse. Three isolates with strong, moderate, and weak effects on seedling emergence were selected and studied in a field experiment under different soil moisture conditions. The results showed that the isolates kept their relative ability to affect seedling emergence in the same manner as observed before, and the harmful effects of the pathogenic isolates increased under dry soil conditions.

Additional experiments were conducted to evaluate seed quality and the behavior of Phomopsis spp. during storage. Naturally heavily infected seeds of 'Parana' and 'Bossier' soybeans were stored in paper bags under ambient conditions at the CNPS seed laboratory and analyzed at 3-month intervals, comparing treated and nontreated samples. In June, the nontreated seed of both cultivars showed a high incidence of Phomopsis spp. and poor germination in the laboratory. After 3 months of storage (September), the percentage of Phomopsis-infected seeds dropped drastically and germination improved from 54.7 to 82.1 percent for the Parana seeds. However, this increase in germination was not observed with Bossier seeds, because, in addition to Phomopsis spp., there was a high incidence of weathering and mechanical damage, as determined by the tetrazolium test.

In conclusion, under the conditions mentioned above, seedborne Phomopsis spp. have not been shown to affect seedling emergence in the greenhouse or field when soil moisture and temperature are adequate. The results have been similar in the germination of fungicide-treated seeds in the laboratory. The fungus is mostly confined to the seedcoat; and upon germination, the cotyledons leave the infected seedcoat in the soil, thus escaping from the pathogen.

Furthermore, it was demonstrated that the fungus loses its viability quite rapidly during storage at ambient conditions, and germination increases gradually. This increase in germination also depends upon the physiological quality of the seed. Mechanical damage, weathering, and stinkbug injury are frequently responsible for reduced seed quality and sometimes are associated with Phomopsis infection. In such cases, even if the fungus has lost its viability, the germination will not reach the minimum standards for sale.

Seed treatment with fungicides before or during storage is not necessary, but it is recommended when planting has to be done under unfavorable soil conditions.

SESSION D. CONTROL MEASURES

Al Y. Chambers, Chairman[1]

Evaluation of the Kentucky Point System for Scheduling the Application of Benomyl on Soybeans Grown for Seed

R. E. Stuckey[2], L. J. Tomes[3], D. M. TeKrony[3], and D. B. Egli[3]

It has been shown that a single application of the foliar fungicide benomyl at growth stage R6 (green pod, full seed) will improve the seed quality of soybeans that are infected with the pod and stem blight fungus. A 24C State label for the use of benomyl by soybean seed growers in Kentucky was approved in 1980. A point system was developed to assist seed producers in deciding when to use a foliar fungicide (table 1).

In the summer of 1982, 19 commercial fields and University test plots were evaluated for response to benomyl applied at the rate of 1.12 kg/ha at the R6 growth stage. The Kentucky point system more accurately predicted those fields that would benefit from fungicide application than other point systems developed in other States and by industry. All fields (5) that had sufficient points for fungicide application had high levels of seed infection by Phomopsis sp., and all fields that had insufficient points resulted in low seed infection levels (table 2). Eight of the 19 fields were in the "may be beneficial" category (table 2). When 4 of these fields were placed with the 5 fields that had sufficient points to recommend fungicide application, seed infection ranged from 10 to 47 percent (mean 28 percent), whereas seed infection in the remaining 10 fields with insufficient points ranged from 0 to 19 percent (mean 9 percent). A pod infection test used in Iowa and Illinois as a guide for determining fungicide application to seed fields, was evaluated in 13 fields in this study. The percentage of pods infected with Phomopsis sp. at growth stage R6 was closely related ($r=0.86$) to seed infection by Phomopsis sp. at harvest maturity in the check plots. Assigning a value of two additional points to those fields where collected pods had 50 percent or more pod infection by Phomopsis sp. did not change the recommendation for the "apply" or "do not apply" fungicide fields, but it did clearly delineate the "may be beneficial" fields into "apply" or "do not apply" categories (table 2).

The four parameters of cropping history, cultivar maturity, planting date, and rainfall currently in the Kentucky point system make it easy for the grower to assign point values. From the 19 fields tested in 1982, fields can be identified where omission of any one of the above four criteria would have lessened the reliability of the point system (table 2). The addition of a fifth parameter, pod infection at growth stage R6, although much more difficult and time consuming to assess appears to be warranted for those fields where point totals are not clearly in the "apply" or "do not apply" category.

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Table 1 - Point system: use of foliar fungicides for soybean seed production in Kentucky.[1]

		Points[2]
I. Cropping History		
In soybeans the previous 2 or more years	3	_____
In soybeans the previous year	2	_____
Not in soybeans last year	0	_____
II. Variety Selection		
Early season variety	3	_____
Mid season variety	2	_____
Late season variety - foliar fungicides should not be used on late season varieties.		
III. Planting Date		
Prior to May 20	3	_____
Between May 20 and June 20	2	_____
After June 20	0	_____
IV. Rainfall - Based on existing rainfall and rainfall predictions during seed development and maturation between R2 and R7 stages of growth		
Rainfall below normal	0	_____
Rainfall near normal (0.5 inches)	2	_____
Rainfall above normal	4	_____
	TOTAL	_____

[1]Foliar fungicides not recommended for use in soybean seed production when any of the following 3 conditions exist: (1) Yield potential is less than 20 bu/acre, (2) heavy weed pressure exists, or (3) a late season variety is planted.

[2]Point system: 11 or above, fungicide should be applied; 9 to 10, fungicide may be beneficial; 8 or less, fungicide should not be applied.

Table 2 - Incidence of seed infected by *Phomopsis* sp. in nonsprayed and sprayed areas of soybean fields and point values assessed each field according to cropping history, variety, planting date and rainfall.

FIELD NUMBER	*Phomopsis* sp. Seed Infection (%)		Points Accumulated						
	Check	Benomyl[2]	Crop history	Variety	Planting date	Rainfall	Total Points[3]	Pod test[4]	Revised point totals[5]
1.	47	33	3	2	2	4	11	2	13
2.	40	27	0	3	3	4	10	2	12
3.	37	17	0	3	3	4	10	2	12
4.	35	13	3	3	3	0	9	2	11
5.	32	--	3	2	3	4	12	-	
6.	19	8	3	2	3	4	12	-	
7.	19	10	0	3	2	4	9	2	11
8.	16	14	3	3	2	4	12	2	14
9.	15	27	3	2	2	2	9	-	
10.	13	12	2	2	3	4	11	-	
11.	11	10	0	2	2	4	8	-	
12.	11	6	0	3	3	0	6	0	6
13.	10	7	2	3	3	2	10	0	10
14.	8	0	0	3	2	4	9	0	9
15.	5	2	3	3	2	2	10	0	10
16.	4	2	0	3	3	0	6	-	
17.	1	0	0	3	2	2	7	0	7
18.	1	0	0	2	3	0	5	0	5
19.	0	3	0	2	3	0	5	0	5

[1]Percentage of seed infected with *Phomopsis* sp. on nonsprayed soybean plants.
[2]Percentage of seed infected with *Phomopsis* sp. on soybean plants receiving 1.12 kg/ha of benomyl at the R6 growth stage.
[3]Point system: 8 or less, benomyl application is not warranted; 9 or 10, benomyl may be useful; and 11 or more, benomyl should be applied.
[4]Collection of pods at R6 and determination of pod infection by *Phomopsis* sp.
[5]Revised point system: all seed fields with 11 or more total points are recommended for fungicide application, fields with 10 or less points are not recommended for fungicide application.

Effect of Two Commercial Fungicides on the Incidence of Diaporthe phaseolorum var. caulivora on Susceptible Soybean Cultivars[1]

R. P. Pacumbaba, V. T. Sapra, and L. K. Prom[2]

The soybean, Glycine max (L) Merrill, is a native and ancient cultivated leguminous crop of eastern Asia. Today, soybeans are grown in most parts of the world. It is one of the most important export crops of the United States and is a primary source of vegetable oil and protein. About 90 percent of the soybean meal or cake is used to feed livestock, poultry, and household pets. Eighty percent of the soybean oil is used in margarine, salad oil, cooking oil, and shortening.

The Southern States produced 31.2 percent (711,475,000 bushels) of the Nation's soybean crop in 1982. An estimated 173.5 million bushels of soybean in these States was lost to diseases, and the loss was valued at about $104 billion. Foliar and stem diseases accounted for about 41 percent. One disease of soybean affecting the leaves and stems is stem canker, and it has become an important economic problem in Alabama in the last 3 years. In 1983, an estimated $17 million in yield was lost because of stem canker, and about 30 percent of Alabama's soybean acreage was affected.

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2) Department of Natural Resources and Environmental Studies, Alabama A & M University, Normal, AL 35762.

Stem canker, caused by Diaporthe phaseolorum var. caulivora (Dpc), is a disease of soybean that limits seed development or kills the plant prematurely. There are two types of symptoms observed in the field in northern Alabama. If the plants are affected by Dpc before the flowering stage, the symptoms of the disease are mostly confined to the leaves and consist of a complete necrotic blight affecting entire leaves. If the plants are affected at the flowering stage, the symptoms of the disease are found both on the leaves and stems. The symptoms found on the stem consist of a lesion which enlarges to become a slightly sunken and reddish-brown canker, several centimeters long, that girdles the stem and automatically kills the plants.

The objectives of this study were to test and evaluate several fungicides for the control of Dpc, and to determine whether before or at the flowering stage is the best time for applying these fungicides.

The experiment was started in 1983 at Alabama A & M University's experimental field plots and was continued to the present. Initially, three commercial fungicides that are readily available in the market were chosen. Dyrene (4,6-dichloro-N-(2-chlorophenyl)-1,3,5-triazin-2-amine) is a broad-spectrum fungicide for controlling field, fruit, vegetable, and ornamental diseases. Manzate 200 or mancozeb (manganese ethelenebis-[dithiocarbamate] and zinc) is also a broad-spectrum fungicide for controlling field, vegetable, and fruit crop diseases. Benlate or benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) is likewise a

broad-spectrum fungicide with protectant and eradicant as well as systemic properties. At the time of the experiment, only Dyrene and Manzate 200 were tested in the field. Since no preliminary assays of the effect of these fungicides on Dpc were done in the laboratory, the recommended dosage rates were used. Dyrene was applied at 6 lb/acre and Manzate 200 was applied at 3 lb/acre.

An isolate of Dpc from the Huntsville area was aseptically obtained from an affected stem and maintained in potato dextrose agar acidified with lactic acid to pH 4.5. The plated isolates were incubated in the dark at 25±1°C for 8 days. The inoculum was prepared by placing eight plates of the pathogen with agar in a Waring blender and blending in sterile, distilled water. The mycelial suspension was transferred to a sprayer, and sterile distilled water was added to the 10-gallon mark. This was used to inoculate soybeans in the field. The mycelial suspension consisted of 10^6 fragments per ml, as determined with a hemacytometer.

The susceptible soybean cultivars used were Bragg and Ringaround 701. These were planted in field plots, 3.05 by 5.86 m in a split-block experimental design, and the plantings were replicated three times per treatment. The fungicides were applied 12 weeks after planting, and Dpc was applied after 13 weeks or about R_1 of the reproductive stage of development of soybeans (Walla, W. J. ed. 1979, Soybean disease atlas by the Southern Soybean Disease Workers. Texas A & M University, College Station). This fungicide application was noted as before inoculation with Dpc. In another set, inoculum was applied 13 weeks after planting or at about the same reproductive stage of soybean development, and the fungicides were applied 14 weeks after planting. This fungicide application was noted as after inoculation with Dpc.

Table 1 - Mean of disease incidence of soybean stem canker on susceptible cultivars when Dyrene and Manzate 200 were applied once, before or after inoculation with Diaporthe phaseolorum var. caulivora.

Fungicide used	Before inoculation				After inoculation			
	Treated		Control		Treated		Control	
	B	RA	B	RA	B	RA	B	RA
Dyrene	21a	35c	53b	88d	26a	50c	53b	103d
Manzate 200	24a	39c	53b	88d	33a	54c	53b	103d

B = cv. Bragg, RA = cv. Ringaround 701. Means with the same letters are not significantly different according to Duncan's multiple range test at alpha 0.05.

Statistical analysis of the initial results indicated that Manzate 200 and Dyrene when applied once before inoculation with the stem canker pathogen gave significantly better control of the disease for both cultivars than when the fungicides were applied after inoculation (table 1). Yields of both cultivars were also significantly higher when the fungicides were applied before rather than after inoculation with Dpc (table 2). Although yield increases of susceptible cultivars were noted in this study, the national mean yield average per acre of soybean was still higher. This condition may indicate that the full effect of the fungicides both on disease incidence and on yield was not obtained. Possibly, if the fungicides had been applied several times at the right dosage rate before or after inoculation with the pathogen, effective control of the disease would have been obtained. If a significant reduction of disease incidence could be attained, perhaps an increase in yield could also be possible.

The following are some of the things we plan to do during the growing season of 1984: 1. Assay Dyrene, Manzate 200, and benlate fungicides on Dpc in the laboratory so that the right dosage of each fungicide for the control of Dpc in the field can be achieved. 2. Apply the fungicides at least five times before or after inoculation with Dpc. 3. Evaluate the best fungicides for the control of Dpc.

Table 2 - Mean yield (kg/ha) of susceptible soybean cultivars when Dyrene and Manzate 200 were applied before or after inoculation with *Diaporthe phaseolorum* var. *caulivora*.

Fungicide used	Before Inoculation				After Inoculation			
	Treated		Control		Treated		Control	
	B	RA	B	RA	B	RA	B	RA
Dyrene	1646a	632c	1016b	369d	1398a	293c	985b	172d
Manzate 200	1607a	499c	1016b	369d	1297a	419c	985b	172d

B = cv. Bragg, RA = cv. Ringaround 701. National soybean mean yield in 1981 was 1704.5 kg/ha. Means with the same letters are not significantly different according to Duncan's multiple range test at alpha 0.05.

Effect of *Diaporthe* and Other Seedborne Pathogens on Soybean Seed Germination and Seedling Survival

J. K. Springer[1] and P. M. Halisky[2]

Foliarly applied fungicides have generally resulted in significant control of pod and stem blight and purple seedcoat, but they have not reduced the levels of *Alternaria* seed decay or downy mildew. Further, the germination of field-run samples from fungicide sprayed plots has not been consistently higher than that from control plots. Thus, studies were initiated to evaluate the effect of seedborne fungi on germination and seedling survival.

Seedborne diseases were quite prevalent in 1977. Percent visually infected seed in control plots was 43 percent pod and stem blight, 7 percent *Alternaria* seed decay, 7 percent purple seedcoat, and 6 percent purple stain. Corresponding percent germination for these seed was 0, 2, 49 and 13 percent. Visually healthy seed from the same seed lot had a 68 percent germination, while seed which exhibited a slight fissuring of the seedcoat had 49 percent germination.

Thus, the germination of seed infected with the pod and stem blight fungus was reduced 28 percent when the seed were only partially colonized, while germination was reduced 100 percent when seed were extensively colonized by the fungus. A similar relationship with the other diseases present could not be determined.

In 1983, no foliarly applied fungicide treatment improved germination, seedling vigor, or damping-off control. No fungicide provided significantly more control of pod and stem blight, but Topsin M was significantly less effective than any of the other fungicides.

Seed were segregated into groups of healthy, pod and stem blight infected, *Alternaria* seed decay infected and downy mildew infected. Germination percent of the corresponding groups was 95a, 81b, 48c and 44c, while the percent of seedlings which would survive in a field planting was 93a, 79b, 40c and 43c.

Visual examination of seed infected with pod and stem blight and *Alternaria* seed decay indicated the individual seed were not highly colonized. At this level of seed colonization, germination was reduced 15 percent by pod and stem blight and 49 percent by *Alternaria* seed decay. Germination of seed infected with downy mildew was reduced by 54 percent. Percent vigorous seedlings correlated very closely with percent germination.

The effect of downy mildew infection on germination was not investigated prior to this year. Consequently, data secured in this study require confirmation.

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Effects of Timing and Rates of Fungicide Applications to Control Stem Canker of Soybeans

Paul A. Backman[1]

Research to chemically control stem canker of soybeans has continued since 1980. Data developed during the first year indicated that applications made at R_3 and R_5 gave moderate disease suppression and yield increases if the fungicide was systemic (benomyl or Baycor) and was accompanied by an oil-surfactant adjuvant. These data indicated that the organism was probably already inside the plant and that systemic activity and improved penetration were necessary for disease control. Later tests proved that disease control was improved if vegetative sprays were applied between V_2 and V_{10}. Again, disease control was improved if subsequent R_3 and R_5 fungicide applications (benomyl) were made. Results from 1983 indicated that applications of benomyl made between V_2 and V_6 were required for adequate disease control in varieties of intermediate susceptibility. As in previous studies, these early sprays were aided by later applications, applications which when used alone were noneffective. Control of stem canker in highly susceptible varieties was impossible, even when as many as five applications were made. Data reported here (table 1, figs. 1 through 4) were developed primarily using systemic fungicides (principally benomyl). Research results from Tennessee (A. Y. Chambers, Stem Canker Conference, Birmingham, AL 1983) indicate that protectant fungicides applied in the early vegetative period (V_2 to V_6) also can control stem canker.

Table 1 - Pretransformed arc-sine rating scale for assessing stem canker damage in soybeans.

Rating*	Disease level	Rating*	Disease level
0	No disease	3.0	65% dead or dying
.1	1 dead plant/30 m	3.5	80% dead or dying
.5	6-8 dead plants/30 m	4.0	90% dead or dying
1.0	10% dead or dying	4.5	6-8 live plants/30 m
1.5	20% dead or dying	4.9	1 live plant/30 m
2.0	35% dead or dying	5.0	All plants dead
2.5	50% dead or dying		

*Ratings intermediate on the 0 to 5 scale should be used if intermediate disease levels can be discerned.

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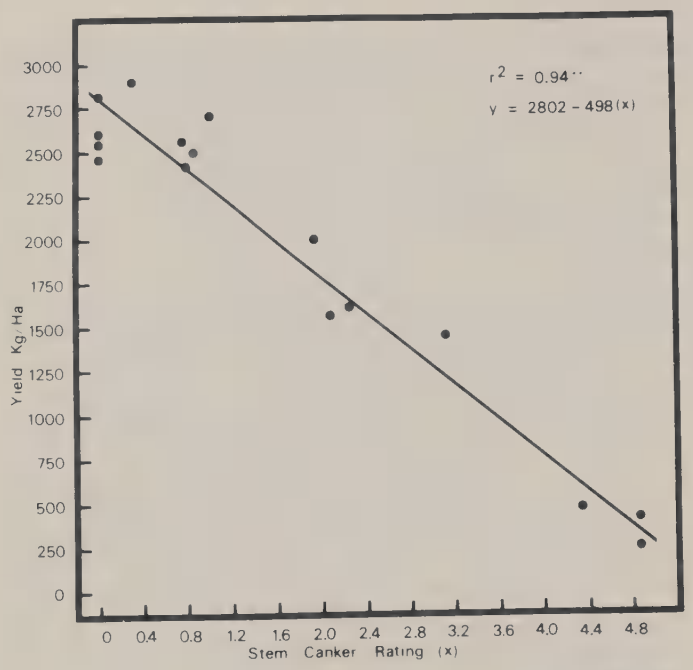

Figure 1.--Relationship between soybean yield and stem canker severity, where the pretransformed arc-sine disease rating system was used.

Rates of application have also been evaluated. For benomyl, 2 oz per acre (Benlate® 50WP) applied in a 30-cm band over the row can give control equivalent to 8 oz applied broadcast. Applications of 16 oz of Benlate® 50WP are only slightly superior to applications of 8 oz. Control is usually improved by the addition of oil-surfactant blends, though if rainfall follows soon after the fungicide application, performance is compromised by the adjuvant.

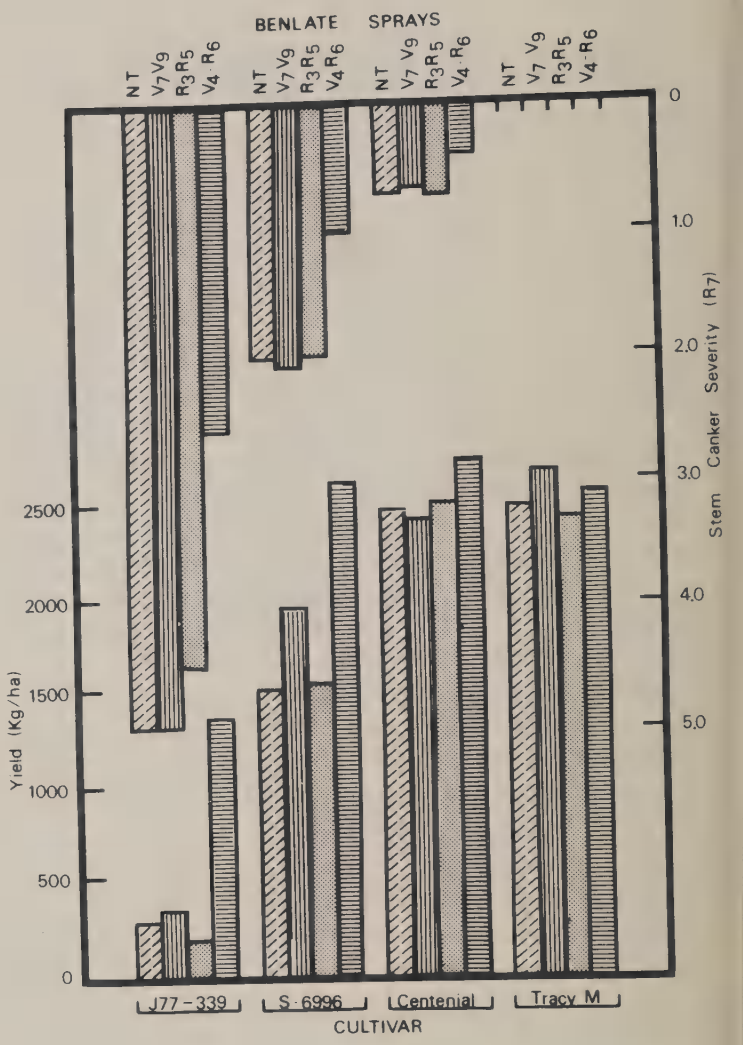

Figure 2.--Yields and stem canker severity of 4 soybean cultivars sprayed with the fungicide Benlate® 50 WP, 0.56 kg/ha (benomyl) on each of 4 different spray schedules. NT refers to a no treatment schedule, and stem canker severity was rated using the pretransformed arc-sine method; yields and disease are plotted in opposition to illustrate their reciprocal nature.

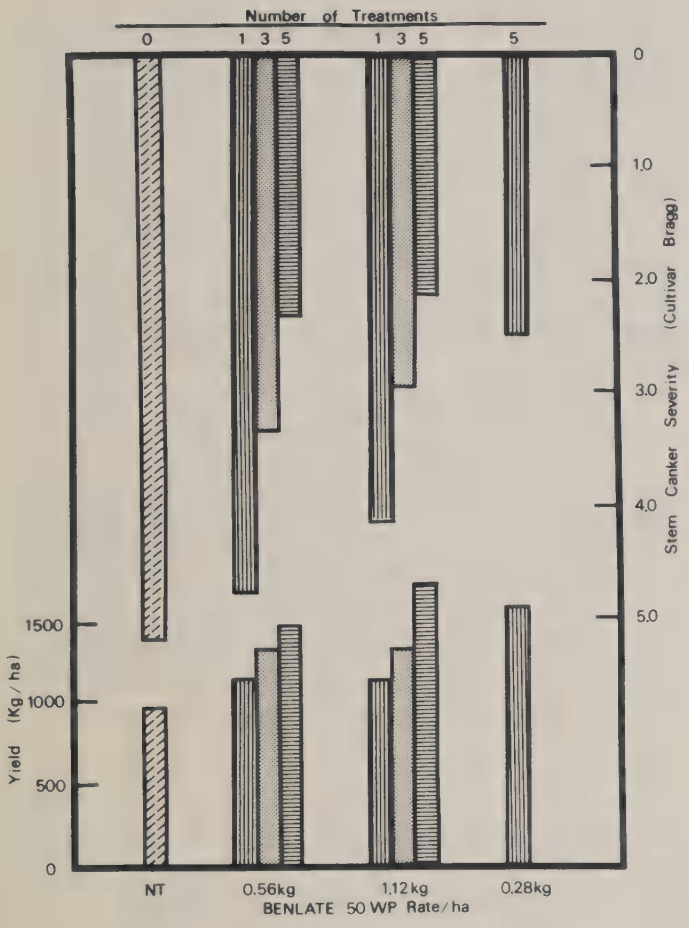

Figure 3.--Yields and stem canker severity of Bragg cultivar soybeans sprayed at various rates either 1, 2, or 3 times. Timings were 1=V_2, 3=$V_2R_3R_5$, and 5=$V_2V_7V_9R_3R_5$.

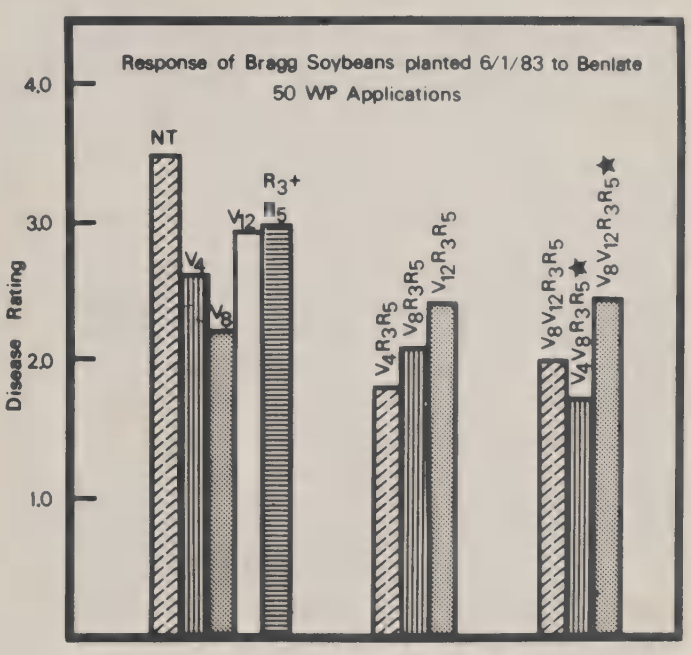

Figure 4.--Effect of one of more applications of benomyl (Benlate® 50 WP) applied at 1.12 kg/ha (*=0.56 kg/ha) at various application timings.

Seed Treatments and Tillage Practices as They Affect Spread and Control of Stem Canker

Mark A. Crawford[1]

Stem canker, caused by Diaporthe Phaseolorum var. caulivora (Dpc), has been a very serious disease in Alabama and the Southeast since the late 1970's. The organism is known to be seed transmitted, and seed are believed to be the way the organism was first introduced into the Southeast. In an effort to control the spread of Dpc from infested to noninfested areas, fungicides commonly used to control seed and seedling diseases of soybeans were evaluated for the control of seedborne Dpc.

Seed found to be 2.5 percent infected with Dpc were treated with the following fungicides: Carboxin (Vitavax), thiram (Gustafson 42-S), captan (Captan 400 D), carboxin plus thiram (Vitavax 200), carboxin plus captan, and carboxin plus thiram plus captan. The nontreated control was surface disinfested with 0.1 percent sodium hypochlorite for 30 sec. Seed were placed in paper-roll dolls, 50 seed/roll doll, replicated 3 times, and kept at 25°C for 3 days. Germination data were taken, following which the germinated seed were surface disinfested for 45 sec in 0.05 percent sodium hypochlorite, followed by 10 sec in 95 percent ethanol, and finally washed in sterile water after removing the seedcoat and radicles. One hundred germinated seed of each treatment and a dry seed control were plated on potato dextrose agar (PDA), pH 4.5, and incubated at 26°C for 14 days. All fungi from seed were identified by morphological characteristics. The growth of Phomopsis spp. was used as a criterion for determining Dpc control since the two fungi are closely related. Neither Dpc nor Phomopsis spp. were found on seed treated with either carboxin or captan (table 1); however, they were detected when these fungicides were combined, and Phomopsis spp. was detected from seed treated with carboxin plus thiram. When seed treated with carboxin plus thiram plus captan were evaluated, they were found to be free of Dpc and Phomopsis spp. (table 2). Fungicides did not improve germination in the first test (table 1); however, a 10 to 20 percent improvement in germination occurred when the test was repeated (table 2).

With such low levels of seed infected with Dpc (<5 percent) and a small sample size (100 seed), the probability of detecting Dpc is low. Similarly, when the probability of detecting Dpc in a seed lot is compared at various sample sizes and at different levels of infection (table 3), there is a 16 percent probability of not detecting Dpc in a seed lot containing a 1 percent level of Dpc when the sample size is 100. If the sample size is increased to 400 seed, this probability of a false negative test is reduced to 2 percent. Since Dpc seed infection levels are only rarely observed to be over 1 percent, it is important to use a sample size greater than 200 seed to assure a high probability of detecting the organism.

Comparisons between conventional and no-tillage cultivation systems indicated that soybeans grown under conventional tillage practices had a higher incidence of stem canker (table 4).

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One possible explanation for this is that a well-developed canopy in the conventional tillage plots favored the development of stem canker. An additional circumstance was the fact that there was no in-test inoculum; all inoculum came from nearby fields. These data differ markedly from results of experiments conducted in Tennessee, where inoculum preexisted in the test area (Chambers 1983).

In conclusion, seed treatment fungicides have been shown to be effective in reducing seedborne Diaporthe phaseolorum var. caulivora. However, since these fungicides do not give complete coverage to every seed, there are escapes. Therefore, treating infected seed is not a fully effective way of preventing the contamination of "clean" land.

Table 1 - Effect of fungicide seed treatments on soybean seed infected with Diaporthe phaseolorum var. caulivora[1]: first test.

Treatment	Rate[2] (oz/cwt)	Dpc %	Phomopsis %	Germination %
1. Control	--	3	1	82
2. Carboxin + thiram	2 + 2	0	1	77
3. Thiram	2	2	2	87
4. Captan 400 D	2	0	0	83
5. Carboxin	2	0	0	84
6. Captan 400 D + carboxin	2 + 2	1	2	86
7. Dry seed	--	2	7	--

[1]Seed lot found to be 2.5 percent infected with Dpc.
[2]Water added to each fungicide to adjust volume to 8 fl oz/cwt.

Table 2 - Effect of fungicide seed treatments on soybean seed infected with *Diaporthe phaseolorum* var. *caulivora*[1]: second test.

Treatment	Rate[2] (oz/cwt)	Dpc %	*Phomopsis* %	Germination %
1. Control	--	3	11	82
2. Carboxin + thiram	2 + 2	0	2	86
3. Thiram	2	0	2	88
4. Carboxin - captan (20-20)	4	0	1	78
5. Carboxin + thiram + captan 400 D	2+2+2	0	0	84
6. Dry seed[3]	--	1.3	13.3	--

[1]Seed lot found to be 2.5 percent with Dpc.
[2]Water added to each fungicide to adjust volume to 8 fl oz/cwt.
[3]300-seed sample.

Table 3 - Probability of detecting *Diaporthe phaseolorum* var. *caulivora* at various levels of seed infection in 3 different sample sizes.

% Infection P (X>0)	Probability*	Sample size (N) 100	200	400
0.1	P	0.6231	0.6725	0.7348
	1-P	.3769	.3275	.2652
.5	P	.7584	.8419	.9207
	1-P	.2416	.1581	.0793
1.0	P	.8416	.9223	.9778
	1-P	.1587	.0777	.0222
2.0	P	.9224	.9783	.9978
	1-P	.0776	.0217	.0022
4.0	P	.9793	.9980	>.9999
	1-P	.0207	.0020	<.0001

P = probability of detection if Dpc is present and 1 - P = probability of reporting a false negative if Dpc is present.

Calculate Z and obtain P from probability table.

$Z = \frac{O-U}{\sigma}$ $U = P(N)$ $\sigma = Npq$

Source: Values obtained from Table A.4, "Principles and Procedures of Statistics" by R. Steel and J. Torrie, 1960.

Table 4 - Effect of tillage methods on the incidence of stem canker and seed yield on 4 soybean cultivars.

Cultivar	Tillage[2]						Mean	
	No till		No till-subsoil		Conventional			
	SCR[1]	Yield (bu/acre)	SCR	Yield	SCR	Yield	SCR	Yield
Coker 237	1.1	34.4	1.2	36.3	1.8	35.5	1.37	35.4
Coker 338	1.8	29.4	2.3	30.5	3.3	26.9	2.47	28.9
Coker 488	1.2	35.3	1.1	36.0	1.2	34.5	1.16	35.3
Hutton	2.5	27.2	3.4	26.6	4.1	21.4	3.33	25.1
Mean	1.65	31.6	2.0	32.4	2.6	29.5		

[1]SCR = stem canker rating. Rating Scale: 1.0 percent, no disease observed; 2.0, 12 percent of plants dead or dying; 3.0, 25 percent of plants dead or dying; 4.0, 50 percent of plants dead or dying; 5.0, 100 percent of plants dead.

[2] All tillage methods followed wheat.

Cultivar and Planting Date Effects on Field Infestations of Soybean Stem Canker

David B. Weaver[1]

Stem canker of soybean caused by Diaporthe phaseolorum var. caulivora is capable of causing severe losses and in recent years has become epiphytotic in areas of the Southeastern United States. There have been reports (2, 4) that the disease appears to be much more severe on some cultivars than on others. Our main objectives were to evaluate several adapted soybean cultivars for reaction to stem canker, based both on disease development and seed yield under conditions of natural infestation. Secondary objectives were to determine the effect of disease severity on yield in a wide range of genotypes and to determine the effect of delayed planting on disease development and yield loss.

Forty-seven cultivars were evaluated in seven experiments during 1982 and 1983. Four-row plots arranged in a randomized complete block design with either three or four replications were rated for disease development at approximately the R6 development stage. Plots were rated visually on the percentage of dead or dying plants, and data were transformed to a 0 to 5 scale based on one-fifth the arc-sine transformation from 0° to 90°, where 0=no disease, 1=10 percent dead or dying plants, 2=35 percent, 3=65 percent, 4=90 percent and 5=100 percent dead or dying plants. Disease development was estimated to the nearest 5 percent and transformed accordingly; for example, 25 percent damage was rated 1.6. Yield data were collected by harvesting the end-trimmed center two rows of each plot. In three locations, planting date effects were evaluated by planting two experiments side by side, one approximately on 15 May and the second approximately on 15 June.

Significant disease development occurred in all experiments, ranging from mean ratings of 0.7 to 1.5. Seed yields and disease ratings of selected cultivars common to four environments are shown in table 1. Only two cultivars, Tracy M and Braxton, had superior resistance. 'Tracy M' showed no disease symptoms, and 'Braxton' had only slight disease development in one environment. 'Braxton' was superior to all other cultivars in seed yield, with a mean of 3,062 kg/ha. Yield of 'Tracy M' was 2,592 kg/ha, but other cultivars, such as Davis, Coker 156, Ransom, and Wright, did not differ significantly from 'Tracy M' in yield, even though their mean disease ratings were around 1.0. There are two possible explanations: 'Tracy M' is not well adapted to Alabama growing conditions, or yield is more influenced by other environmental factors at disease levels below 10 percent. 'Bay' was only slightly less resistant than 'Tracy M' and 'Braxton'.

Several cultivars, such as Jeff, Hutton, and Coker 237, were susceptible to stem canker, with disease ratings of 1.5 for 'Jeff' and 'Coker 237' to 2.2 for 'Hutton'. Seed yields were correspondingly low. In production fields, these cultivars have shown damage levels of up to 80 percent in published reports (4) and 100 percent in unpublished reports. The comparatively low ratings (the 2.2 rating for 'Hutton'=40 percent damage) observed in these experiments may have resulted from relatively low disease pressure, or perhaps because the plots

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Table 1 - Seed yields and stem canker ratings of selected soybean cultivars grown in 4 environments during 1982 and 1983.

Cultivar	Seed yield (kg/ha)	Disease rating
AP 70	2499	1.0
Bay	2750	.3
Bedford	2173	1.3
Braxton	3062	.2
Centennial	2531	.7
Cobb	2332	.9
Coker 156	2557	1.0
Coker 237	2236	1.5
Coker 488	2401	.9
Davis	2667	1.0
Deltapine 105	2820	1.0
Essex	2238	M[1]
Forrest	2317	1.2
Foster	2144	1.0
GaSoy 17	2417	1.0
Govan	2340	1.1
Hutton	1071	2.2
Jeff	2107	1.5
Kirby	2102	1.0
Lee 74	2217	1.4
Ransom	2667	1.1
Tracy M	2592	.0
Wilstar 790	1541	1.8
Wright	2638	.8
LSD (0.05)	180	.2

[1]Essex was at physiological maturity when ratings were made.

were in some cases rated too early. The ideal time to rate would be during the early R7 stage. Because the cultivars in these experiments represented a wide range of maturity dates (maturity groups V through VIII), it was necessary to rate at different stages of development. Ratings were made when the early cultivars were approaching maturity. At this time, the late maturing cultivars were still in the R6 development stage.

Based on data from other experiments not shown in table 1, no cultivars were found to be as resistant to stem canker as 'Tracy M' and 'Braxton'. RA 604, RA 702, and RA 801 were found to be susceptible, with mean ratings of 2.0, 2.0, and 2.1, respectively. Observational data on cultivars not included in these experiments suggest that 'Dowling' is resistant and that 'Bragg' and 'Coker 338' are susceptible. Relative resistance or susceptibility of these cultivars is difficult to determine because of the lack of replicated data.

Table 2 - Effect of planting date on seed yields of selected soybean cultivars, 1982 and 1983.

Cultivar	Seed yield (kg/ha)			
	1982[1]		1983	
Resistant	Early	Late[2]	Early	Late
Bay	2562	2267	4009	2523
Tracy M	3004	2478	4047	2614
Centennial	2210	2456	4070	2889
Braxton	2614	2648	4215	3018
$\bar{x}$	2597	2462	4085	2761
Moderately Resistant				
Deltapine 105	2805	2471	4451	2782
Davis	2612	2389	3857	2743
Ransom	1984	2504	4116	3087
Wright	1955	2392	4139	3186
$\bar{x}$	2339	2439	4141	2950
Moderately Susceptible				
Bedford	2230	1890	3788	2835
Forrest	2157	2136	3925	2256
Jeff	2220	2237	3438	2698
Coker 237	1615	2091	3712	2858
Foster	1930	2141	3529	2912
Kirby	1227	2229	3453	2881
$\bar{x}$	1897	2121	3641	2740
Susceptible				
Wilstar 790	642	1868	2378	2995
Hutton	607	1656	1509	2988
$\bar{x}$	625	1762	1944	2992

[1]Data for 1982 represent the mean of 2 experiments; data for 1983 are from 1 experiment only.
[2]Early planting was approximately 15 May, and late planting approximately 15 June.

Effects of delayed planting were compared for several cultivars (table 2). Cultivars were divided into categories based on mean disease ratings, and data are shown for 1982 and 1983 separately to illustrate the effect of years on delayed planting response. Data from 1982 represent the mean yields of both early (15 May) and late (15 June) plantings at two locations. Growing conditions, particularly moisture availability, were favorable for both planting dates in 1982. As a group, resistant cultivars suffered a slight yield reduction from delayed planting; but moderately resistant, moderately susceptible, and susceptible cultivars had yield increases of 4.3, 11.8 and 182 percent, respectively. Response of early maturing (groups V and VI) cultivars to delayed planting was generally less than full season (groups VII and VIII) cultivars. Data from 1983 are from a single location. Yields from the late planting in the second location were extremely low due to extended dry weather shortly after planting, so the data were not included. Dry weather also depressed yields in the late-planted plots at the location shown in table 2, so that yield response was only observed in the susceptible cultivars. Thus, delayed planting may lessen yield loss due to stem canker, but other production factors may negate this effect. It should also be pointed out that disease levels in these tests were relatively low, generally from 10 to 50 percent infected plants per plot, so that benefits from delayed planting may be greater under higher disease pressures.

The interaction between environments and cultivars for disease rating across all locations was significant. This may indicate the presence of different biotypes of the disease-causing organism or could simply be the result of cultivars differing in their response to stem canker under different environmental conditions. There was also a significant planting date x cultivar interaction for disease rating, which may be the result of the confounding factor of differing maturity dates.

If these cultivars can be considered representative of the adapted southern germplasm pool, it is estimated that at least 10 to 25 percent of the available cultivars have levels of susceptibility that seriously affect their yield performance and should be eliminated from production as soon as possible. Some of these cultivars, such as Bragg, Coker 237, and Hutton are popular and have been grown on a large hectareage in the past and will probably continue to be grown by some producers in spite of the threat posed by stem canker. A strong educational effort should be made to convince producers not to grow susceptible cultivars. This may aid in overall control, as the elimination of susceptible types did in the Midwest in the 1950's (1).

The remaining cultivars have at least some degree of genetic resistance and should be managed accordingly. Seed yields were negatively correlated with disease ratings in all experiments, with r^2 values ranging from 0.38 to 0.61. Thus, stem canker accounted for a major portion of the variability in yield among these cultivars.

Results also indicate that several cultivars have high enough levels of resistance to be included in overall disease control programs. There appears to be a relationship between resistance to soybean cyst (Heterodera glycines) and root-knot nematodes (Meloidogyne spp.) and stem canker

susceptibility. Plant breeders need to develop cultivars with both good stem canker resistance and resistance to several nematode species, especially cyst nematodes. The two cultivars most resistant to stem canker, 'Braxton' and 'Tracy M', have no resistance to the soybean cyst nematode. Evidence that physiological races of stem canker exist (3) may complicate efforts by breeders to incorporate resistance into improved types. There may also be danger in utilizing a single source of resistance (for example, 'Tracy M') because of the possibility that biotypes pathogenic to that source may exist. Benefits of delayed planting for stem canker control are dependent upon the relative susceptibility of the cultivar and the late-planting growing conditions. Because of other factors involved in late-planted production that may reduce yields, delayed planting should not be relied upon as a stem canker control measure except when it is necessary to plant a susceptible cultivar.

References

1. Athow, K.L. 1973 Fungal diseases. p. 459-489. In B. E. Caldwell, editor, Soybeans: improvement, production, and uses. Am. Soc. Agron. Monogr. 16, Madison, WI.

2. Backman, P.A., M.A. Crawford, J. White, D.L. Thurlow, and 1981 L.A. Smith. Soybean stem canker: serious disease in Alabama. Auburn Univ. Highlights Agric. Res. 28(4):6.

3. Keeling, B.L. 1984 Evidence for physiologic specialization in Diaporthe phaseolorum var. caulivora. J. Miss. Acad. Sci. Suppl. 29:5.

4. Krauz, J.P. and B.A. Fortnum. 1983 An epiphytotic of Diaporthe stem canker in South Carolina. Plant Dis. 67:1128-1129.

Prevalence and Control of Seedborne Phomopsis of Soybean in Brazil[1]

H.A. Bolkan[2]

Many researchers consider the pod and stem blight caused by Diaporthe phaseolorum var. sojae (Phomopsis sp.) to be the principal disease involved in the loss of seed germination and field emergence in many soybean [(Glycine max (L.) Merr.] production areas. Brazil is the second largest soybean producer in the world, yet research in seed pathology has been somewhat neglected for a long time. Recently, however, increased attention has been given to the important role that micro-organisms can play in reducing seed quality, field emergence, and yield. Thus, it became inevitable to perform seed health tests, evaluate cultivars, and determine the efficacy of fungicides which could be used under Brazilian conditions.

Incidence of Phomopsis

Soybean seedlots from 18 commonly grown cultivars produced in 1975-81 in the States of Goias, Rio Grande do Sul, Sao Paulo, and Federal District were bioassayed during each year of production using both the blotter and the potato dextrose agar (PDA) plate methods. The incidence of seedborne Phomopsis was found to be related to year, growing region, and cultivar assayed. Percentage recovery of Phomopsis differed significantly between but not within cultivars or year of production. Generally, most of the cultivars selected exhibited a relatively low (0.5 to 3 percent) incidence of seed infection with Phomopsis. Percentage Phomopsis incidence, however, was relatively high (up to 78 percent) among seedlots of the cultivars IAC-2, IAC-5202, and IAC-7053 grown in Federal District. Regardless of the cultivars employed or year of assay, Phomopsis was detected with approximately the same frequency from both the seedcoat and the embryo tissue. Of the two methods used, blotters resulted in a significantly higher percentage recovery of seedborne Phomopsis than PDA plates.

In 1981, the 18 cultivars selected were grown under field conditions and attempts were made to isolate Phomopsis from leaves, stems, pods, and seeds throughout the growing season at biweekly intervals, using different media. We found significant differences in percentage Phomopsis recovery among plant parts and cultivars assayed. The fungus was isolated from stems and seeds of most cultivars but not from leaves or pods. The highest incidence of Phomopsis was seen in seeds (75 percent) and stems (3.5 percent) of the cultivar IAC-2. Percentage incidence of Phomopsis among the rest of the cultivars assayed varied from negligible (0.0 to 2 percent) to moderately high (18 to 35 percent). With most cultivars studied the percentage recovery of Phomopsis increased from none at 57 days after planting to its highest level at harvest, suggesting that the incidence of Phomopsis increases with the physiological maturity of the plant. Furthermore, the significant differences found in percentage incidence of Phomopsis among cultivars

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grown under the same climatic conditions suggest the presence of a resistance mechanism in cultivars showing none to negligible infection levels.

Seed Germination and Field Emergence

Studies involving seed germination have shown a high inverse correlation between percentage seed infection with Phomopsis and percentage seed germination and seedling emergence in the field. As the percentage of seedborne Phomopsis increased, the percentage seed germination in vitro and field emergence decreased.

Fungicide Seed Treatment

Benomyl, captan, and thiram were evaluated as seed treatments, using seeds of cultivar IAC-2 showing 78 percent natural infection with Phomopsis. Seeds were treated with a slurry of benomyl, captan, or thiram at the rates of 30, 20 and 35 mg a.i./20 g of seed and were planted in soil previously steamed at 70°C for 20 minutes or in unsteamed soil under field conditions. All fungicide treatments significantly increased percentage seed germination and seedling emergence compared to untreated control seeds. Similarly, seeds harvested from plants grown from fungicide-treated seeds had significantly less seedborne Phomopsis than seeds from plants grown as controls. These results suggest that the fungicides were capable of moving through the seedcoat to control Phomopsis which was located within the seedcoat and/or embryo tissue; thus providing a beneficial effect to poor quality seeds.

Fungicide Sprays

Benomyl, thiabendazole, thiophanate-methyl, chlorothalonil, and captafol were evaluated as foliar sprays at the rates of 555, 555, 555, 833, and 276 g a.i./ha, respectively. Each fungicide was applied twice in 800 L/ha water--the first at 37 days following planting and the second at early pod stage. All fungicides significantly reduced the percentage incidence of Phomopsis in both the seedcoat and the embryo tissue, and significantly increased percentage seedling emergence in the field. Of the fungicides evaluated, benomyl provided the highest reduction in seedborne Phomopsis. All fungicides increase yields; however, only captafol, thiophanate-methyl and thiabendazole gave significantly higher yields per hectare than the untreated control plots.

Microbial Antagonism and Soybean Seed Quality

In 1982, we initiated greenhouse tests to study the antagonistic effect of Bacillus subtilis, Trichoderma harzianum, T. viride, and an unidentified species of Pseudomonas on soybean seed quality. Our preliminary results showed that seed quality (that is, seed germination and seedling emergence) was not affected when seeds were treated with any of these organisms. In some cases, seeds treated with B. subtilis or Pseudomonas sp. showed a decrease in percentage germination. However, when the soil was infested with the test organisms 3 days prior to planting, an increase in percentage seed germination, seedling emergence, and fresh weight of seedlings was observed. Studies to further explore the influence of microbial antagonism on soybean seed quality are being conducted.